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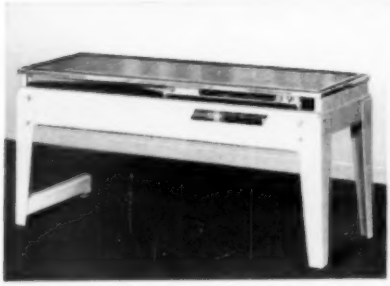

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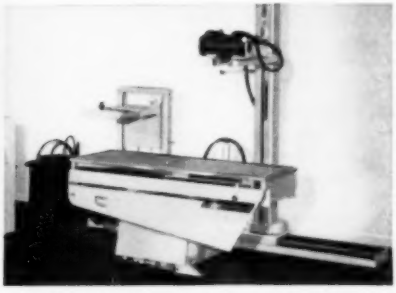

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VOLUME 65 *May 1952* NUMBER 5

THE USE OF BASAL RESPIRATORY MINUTE VOLUME AS AN INDEX OF IMPAIRED PULMONARY FUNCTION[1]

ROBERT S. ALEXANDER

(Received for publication January 11, 1952)

INTRODUCTION

The functional requirements of the body demand that its various physiologic mechanisms be capable of maintaining an efficient basal activity and also have a large functional reserve available to be called forth under conditions of stress. In general, disease processes act to impair the efficiency of the basal function as well as to restrict the maximal functional capacity. Because of the difficulties of correctly standardizing functional tests under stress, physiologic impairment is frequently evaluated in terms of a disturbance in the efficiency of the basal function.

In the specific instance of pulmonary ventilation, an evaluation of basal function is complicated by the fact that respiration is exceedingly susceptible to psychic influences. Not only do individual breathing patterns exhibit striking variations (1), but the actual minute volume of ventilation of a subject may be markedly elevated by recall of situations with emotional content (2, 3). Recognition of the difficulties of excluding psychic factors has prompted the search for other tests of respiratory function. Many writers still make use of the basal ventilation rate, however, without either defending or questioning its validity. It therefore seemed of value to compare data obtained from normal persons with those suffering from known pulmonary disease to ascertain whether there was any justification in employing the basal respiratory minute volume as an index to the impairment of pulmonary function.

MATERIAL

Data for basal respiration of normal subjects were obtained from routine basal metabolism recordings. From a group of 85 healthy medical students without any history of pulmonary disease who were taking basal metabolism tests as part of their physiology course, 61 recordings were selected in which the respiratory pattern was sufficiently uniform throughout the recording period so that the

[1] From the Medical Research Laboratory, Brecksville Veterans Administration Hospital, Brecksville, Ohio, and the Department of Physiology, School of Medicine, Western Reserve University, Cleveland, Ohio.

basal minute volume could be fairly accurately estimated from the average tidal volume and the respiratory rate. An additional 39 cases were selected in the same manner from routine basal metabolism tests on hospital patients[2] who had no history of pulmonary or cardiac disease and whose basal metabolic rates were found to be within 5 per cent of average normal.

The two groups were compared with a series of 100 afebrile patients with pulmonary tuberculosis. These patients had been referred to the laboratory to evaluate their suitability for collapse procedures and, therefore, in the majority of instances were suffering from far advanced pulmonary tuberculosis. The basal ventilation in these patients was recorded in the same manner as in the usual basal metabolism test except that it did not appear relevant to this study to have the patients strictly postabsorptive. The spirometer employed was equipped with a ventilometer (4) so that minor irregularities in tidal volume were of no significance in calculating the respiratory minute volume. No record was considered, however, in which the minute volume did not remain constant for at least four minutes.

RESULTS

To correct for the different basal requirements of the individual subjects, the data were expressed in terms of a ventilation equivalent: the amount of ventilation required per liter of oxygen consumed. The mean ventilation equivalent for the 100 normal persons was thereby found to be 32.1 $\pm$ 10.4 liters while that of the 100 patients with pulmonary tuberculosis was 32.4 $\pm$ 9.1 liters. Obviously there is no significant difference in the values obtained from these two populations, as may be visualized by the distribution of the data shown in figure 1.

It should be recognized that many of the tuberculous patients had lesions which did not involve a dominant portion of the pulmonary parenchyma. Especially in the case of apical disease, fairly extensive infiltration and cavitation may be present without significantly disturbing the function of the lower portions of the lung which appear to make the major contribution to basal respiration. On one occasion, therefore, the hospital population was surveyed and 5 patients were selected who exhibited the most obvious indications of pulmonary insufficiency.

These were patients with terminal illnesses with massive bilateral disease, all of whom exhibited some respiratory distress and cyanosis. Four of these patients died of terminal pulmonary tuberculosis within one month; the fifth died approximately three months later. Ventilation equivalents of these patients ranged from 38.9 to 47.5 liters, with a mean of 42.8 liters.

On the basis of a normal distribution curve (without considering some tendencies for skewness which are suggested in figure 1), approximately one-sixth of the normal population would be expected to have a value as high or higher than the lowest value observed in these terminal patients; the highest value recorded in this terminal group would still be equaled or exceeded by one-twelfth of the nor-

[2] We are indebted to Dr. J. M. Hayman of the Department of Medicine, Western Reserve University, who kindly made these records available.

mal population. There is little doubt that, with sufficient accumulation of data, there could be demonstrated a significant elevation of the mean ventilation equivalent in populations with frank pulmonary insufficiency as compared with the normal population. The important point to be considered here, however, is that when considered as isolated cases not one of these patients with terminal pulmonary tuberculosis could be reliably distinguished from the normal population on the basis of the efficiency of their basal respiration.

Inspection of the original data indicated a rough correlation between ventilation equivalents and respiratory rate. It therefore appeared possible that part of the extreme normal variation might be related to excessive dead space ventilation in the persons exhibiting the more rapid respiratory rates. Failure to correct for this dead space ventilation would obviously give a distorted impression of the efficiency of pulmonary gas exchange.

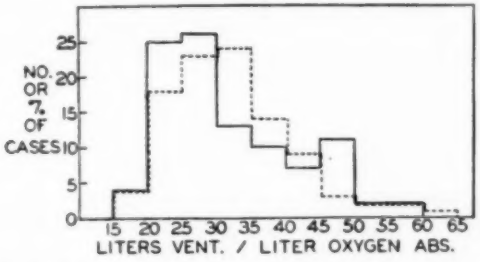

FIG. 1. Distribution curves for ventilation equivalents in 100 normal subjects (solid line) compared with 100 patients with pulmonary tuberculosis (broken line).

Preliminary attempts were therefore made to correct the data for dead space ventilation, using various criteria for dead space that have been proposed (5). When a plot of all the normal data was made as in figure 2, however, the futility of such a calculation at once became apparent. It will be observed that in normal subjects a doubling of the respiratory rate results in an approximate doubling of ventilation per liter of oxygen absorbed. As an alternative to the untenable inference that virtually 100 per cent of the excess ventilation was dead space, it must be concluded that the normal persons who had the high ventilation equivalents were exhibiting true physiologic hyperventilation, presumably associated with the psychic stimulus of the testing procedure. In two instances where a marked hyperventilation was observed, the subjects were kept connected with the spirometer for extended periods to determine whether the hyperventilation would not eventually subside. Both recordings had to be eventually terminated when the subjects exhibited mild convulsions due to acute respiratory alkalosis. Evidence that a significant acapnea develops with psychogenic hyperventilation has been presented by others (6).

A majority of the determinations reported here involved initial tests. The recorded basal minute volumes may have been distorted by the factor of appre-

hension to a strange procedure, in spite of the fact that a good portion of the normal "hyperventilators" were in the medical student group in whom such apprehension should be minimal. Retesting of a number of patients, however, has failed to demonstrate that a retesting procedure will eliminate more than a minority of the hyperventilators. One patient was retested on four different occasions but consistently exhibited basal minute volumes of more than 13 liters per minute with ventilation equivalents between 40 and 50 liters; yet an intensive series of other laboratory tests failed to reveal any physiologic basis for this abnormal respiratory behavior. The explanation for these high values was obtained by carefully observing the patient during the testing procedure. A definite

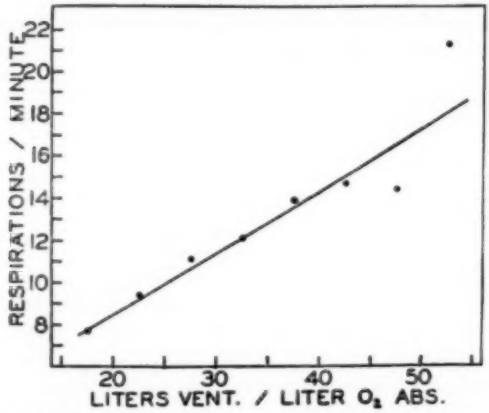

FIG. 2. Relationship between respiratory rate and ventilation equivalent in normal subjects; points show mean respiratory rates for each class of ventilation equivalents as shown in figure 1.

hyperpnea was elicited the moment when the patient was presented with a face mask or mouthpiece before he was connected with the spirometer. Repeated tests did not allay this reaction.

DISCUSSION

The data obtained in this study re-emphasize the susceptibility of the respiratory system to psychogenic influences and indicate the impossibility of uniformly obtaining psychologically basal conditions in performing routine determinations of basal pulmonary ventilation. It is conceivable that, by taking more elaborate precautions with their subjects, others might be able to achieve a closer approximation to basal conditions than existed in the present study. It must be emphasized, however, that until others have proved such an ability to achieve truly basal respiration in their subjects, they have little justification to use measurements of basal respiratory minute volume as a valid index to pulmonary function.

The implications of this study apply, not only to basal respiratory minute volume per se, but also to any other estimation of pulmonary function which depends, in part, upon the basal respiration. These would include ventilation equivalents, as discussed above, and also the basal breathing reserve as calculated from the basal ventilation and the maximum breathing capacity. In addition, many methods for alveolar air analysis and for residual air volume determination involve the implicit assumption that respiration immediately prior to or during the testing procedure was proceeding at a true basal level. In determinations of this type, statistical validity obtained by studying mean trends in a large series of cases should never be used as a basis for the fallacious assumption that such validity may be transferred to the problem of evaluating individual patients.

It is nevertheless becoming progressively more important that adequate pulmonary testing procedures be available to be applied to the individual case to serve as a guide to diagnosis and therapy. The data presented here underscore the challenge to develop more adequate testing procedures for this type of application. It is possible that such tests as those involving a study of "walking ventilation" (7) may involve relatively less psychic distortion of actual functional capacities, although definite evidence to establish such an assumption is needed.

SUMMARY

Basal respiratory minute volumes were determined upon 100 persons without evidence of pulmonary or cardiac disease and upon 100 patients with pulmonary tuberculosis. There was no statistically significant difference between the respiration of the normal population and of the diseased population when analyzed on the basis of the liters of basal ventilation for each liter of oxygen consumption. Five patients with terminal pulmonary tuberculosis and obvious signs of pulmonary insufficiency exhibited ventilations that were within the upper range of normal variability. It is assumed that psychogenic factors played a large role in accounting for the wide variation observed in the normal population. On the basis of this evidence, it is concluded that the determination of the basal respiratory minute volume or any other pulmonary function test that depends upon basal respiration cannot be used validly in the routine estimation of the functional status of individual patients.

SUMARIO

Empleo del Volumen-Minuto-Respiratorio-Basal como Indice de la Disfunción Pulmonar

El volumen de la respiración basal por minuto fué determinado en 100 personas sin signos de afección pulmonar o cardíaca y en 100 tuberculosos pulmonares. Analizada a base de los litros de ventilación basal por litro de consumo de oxígeno, no hubo diferencia de importancia estadística entre la respiración de la población normal y la población enferma. Cinco enfermos con tuberculosis pulmonar terminal y signos manifiestos de insuficiencia pulmonar revelaron ventilaciones que quedaban en los límites superiores de la variabilidad normal. Se da por sentado que los factores psicógenos desempeñaron un gran papel en la amplia variación observada en la población normal. A base de estos datos, dedúcese que no cabe usar, en forma válida, ni la determinación del volumen-minuto-respiratorio-basal ni ninguna otra prueba de la función pulmonar que utilice la respiración basal, para la valoración sistemática del estado funcional de un sujeto dado.

RESUME

Le volume respiratoire basal par minute, comme indice de l'altération de la fonction pulmonaire

Les volumes respiratoires de base par minute furent déterminés chez 100 sujets indemnes de signes d'affections pulmonaires ou cardiaques, et chez 100 malades atteints de tuberculose pulmonaire. Du point de vue statistique, il n'existait pas de différence significative entre la respiration dans la population normale et dans le groupe des individus malades, quand cette fonction a été etudieé sur la base du volume de la ventilation de base par rapport à celui de la consommation d'oxygène. Chez cinq malades, au stade terminal de la tuberculose pulmonaire et présentant des signes évidents d'insuffisance pulmonaire, la ventilation demeurait au niveau supérieur des variations normales. L'auteur considère que les facteurs psychogènes ont joué un rôle important dans les variations considérables observées dans la population normale. D'après ces observations, la conclusion de cette étude est que la détermination du volume respiratoire basal par minute, ou toute autre épreuve de la fonction pulmonaire reposant sur la respiration basale, ne peut être utilisée de façon valable pour l'évaluation routinière de l'état fonctionnel particulier de chaque malade.

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THE EFFECT OF PYRAZINAMIDE (ALDINAMIDE[1]) ON EXPERIMENTAL TUBERCULOSIS IN MICE[2]

LUKE MALONE, ALLAN SCHURR, HOWARD LINDH, DORIS McKENZIE, J. S. KISER, AND J. H. WILLIAMS

(Received for publication February 18, 1952)

INTRODUCTION

The effect of nicotinamide on experimental tuberculosis of white mice was reported by McKenzie and her associates (1). In a continuation of these studies, pyrazinamide (Aldinamide) was prepared by Kushner and co-workers (2). This compound has been tested over a period of nearly three years to evaluate its activity against experimental tuberculosis in mice. During this time the test described by McKenzie and associates has undergone a number of modifications intended to increase the objectivity of evaluation and to decrease the labor involved in the test, without sacrificing sensitivity. The original test, based on that described by Youmans and McCarter (3), used gross appearance of the excised lung and the amount of disease present as indices of activity of therapeutic substances. As these criteria necessarily involved somewhat subjective evaluations, the infection was made more acute by increasing the infecting dose slightly and by using as indices of activity the survival time of 50 per cent of the mice (T_{50}) as compared with 50 per cent of nontreated controls, together with the weight of the mice. Recently, time–per cent effect curves (4) have been employed to evaluate the data statistically, thereby further increasing the objectivity of the test.

EXPERIMENTAL METHODS

Male albino mice, CF_1 strain, weighing 20 plus or minus 3 gm. were used in the tests. The mice were each weighed and placed in separate cages. Ten mice were used for each group, and the individual groups had approximately the same weight. The mice were then infected intravenously with 0.3 mg. wet weight of *Mycobacterium tuberculosis* var. *hominis* H37Rv, suspended in 0.2 ml. of phosphate-buffered saline. Included in each experiment were an infected, nontreated control group and a noninfected, nontreated control group, as well as an infected group treated with 0.5 mg. of streptomycin per day injected subcutaneously in a volume of 0.1 ml. of phosphate-buffered saline.

The infected mice were treated with pyrazinamide (Aldinamide), starting on the day of infection. Either the oral or the subcutaneous route of administration was used. For oral treatment the drug was mixed with the diet of ground Rockland mouse pellets. For subcutaneous administration the drug was dissolved in phosphate-buffered saline, in which it is soluble to the extent of approximately 12 mg. per ml.

Five milligrams of pyrazinamide (Aldinamide) suspended in 0.2 ml. of phosphate-buffered saline and injected subcutaneously in the mice were rapidly absorbed with no evi-

[1] Proprietary name, American Cyanamid Company.

[2] From the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

dence of toxicity. A diet containing 2 per cent of the drug was consumed at a rate to give a drug intake of 80 mg. per mouse per day or 4 gm. per kg. per day, with no evidence of toxicity.

The course of the infection was followed by daily observations of the vitality of the mouse, food intake, and excretions. The mice were weighed at weekly intervals. In the earliest tests, gross and microscopic observations of the lungs served as criteria of activity. Later the T_{50} of the treated group compared with that of the infected, nontreated controls was the principal method of evaluating the activity. The T_{50} was the time in days required for 50 per cent of the mice in a group to die. When 50 per cent of the infected, nontreated mice had died, treatment was stopped in the treated groups and the mice were held until a T_{50} was reached. The remaining mice were usually sacrificed. At least half of the mice in each group were autopsied. When the T_{50} of the infected nontreated controls was sixteen to eighteen days, a prolongation of survival time of three days was considered significant.

TABLE 1

MEDIAN SURVIVAL TIME OF NONTREATED AND PYRAZINAMIDE-TREATED GROUPS

EXPERIMENT	NONTREATED		PYRAZINAMIDE 0.1 per PER CENT IN FEED	
	ET_{50}, days	95 Per Cent Confidence Limits	ET_{50}, days	95 Per Cent Confidence Limits
1273B-119	15.7	14.4 –17.2	18.9	17.6 –20.4
1407B-23	14.9	13.0 –17.0	16.9	15.7 –18.2
1407B-143	15.9	14.8 –17.1	17.9	16.2 –20.2
1469B-29	18.1	17.1 –19.1	19.0	17.1 –21.5
1469B-55	17.4	16.2 –18.7	20.0	17.2 –23.2
1469B-71	16.1	14.1 –18.3	19.3	17.0 –21.9
1469B-95	16.8	15.4 –18.3	20.0	16.2 –24.7
1469B-125	18.2	16.5 –20.1	20.2	17.9 –22.8
1469B-147	16.9	14.1 –20.2	20.7	18.0 –23.8
1469B-167	17.0	15.6 –18.6	30.2*	22.0 –41.5
1508B-1	17.0	14.0 –20.6	21.9	18.5 –25.9
1508B-29	18.5	16.7 –20.4	20.0	17.7 –22.6
1508B-47	17.8	16.4 –19.3	21.2	19.0 –23.6
1508B-67	17.2	16.4 –18.1	20.0	17.8 –22.5
Mean	16.96	15.32–18.75	19.69	17.34–22.35

* Omitted from calculation of mean.

The Litchfield method of graphic analysis of time–per cent effect curves and comparisons of weight data are now used criteria of activity.

In brief, the Litchfield method involves the determination of the median survival time (ET_{50}) of control and treated groups by plotting per cent mortality against time, using logarithmic-probability scale paper. The reaction ratio (RR) of the pair of groups is determined by dividing the ET_{50} of the treated by that of the control group. The slope ratio (SR) is determined by dividing the slope of the time-mortality line for the treated group by that for the control group. Next f_{RR} and f_{SR} values are obtained from a nomograph. The value of f_{SR} should exceed the SR if the lines are statistically parallel. The value of RR should exceed that of f_{RR} if the prolongation of survival time of the treated group is significantly greater than that of the control group.

RESULTS

Early experiments established that a level of 0.2 per cent of pyrazinamide (Aldinamide) in the diet exerted antituberculous activity, while 2.5 mg. twice

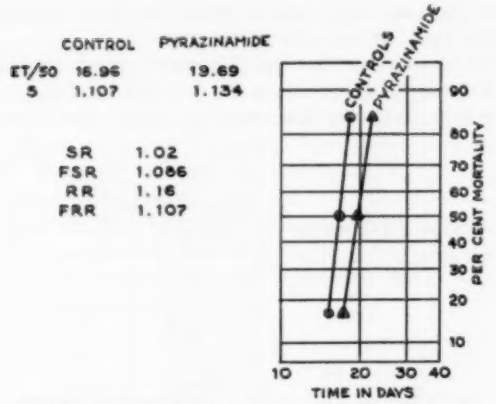

FIG. 1. Average values for control and pyrazinamide-treated mice. Pyrazinamide given in drug diet at 0.1 per cent.

TABLE 2

STATISTICAL ANALYSIS OF NONTREATED AND PYRAZINAMIDE-TREATED GROUPS

	MEAN	STANDARD DEVIATION	95 PER CENT CONFIDENCE LIMITS FOR A SINGLE ITEM	95 PER CENT CONFIDENCE LIMITS FOR MEAN
Nontreated				
ET_{50}	16.96	±1.033	±2.231	±0.596
Slope	1.107	±0.049	±0.106	±0.028
Pyrazinamide (0.1 per cent in feed)				
ET_{50}	19.69	±1.325	±2.888	±0.802
Slope	1.134	±0.044	±0.095	±0.026

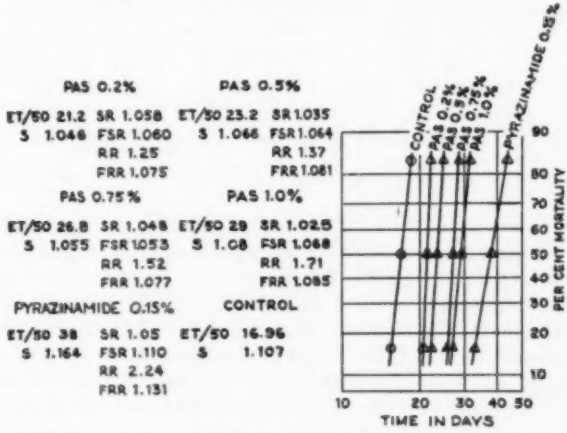

FIG. 2. Comparison of para-aminosalicylic acid (PAS) and pyrazinamide in drug diet.

daily showed minimal activity, based on gross and microscopic examination of the lungs and the T_{50} of treated and control groups.

In table 1 may be seen the ET_{50} and 95 per cent confidence limits for the infected nontreated control group and a group treated with 0.1 per cent pyrazinamide in the feed for each of fourteen consecutive experiments. It also gives

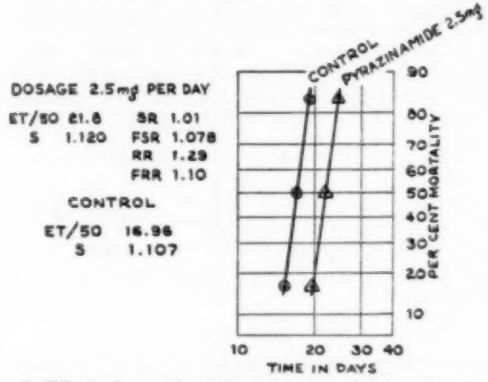

FIG. 3. Effect of pyrazinamide administered subcutaneously.

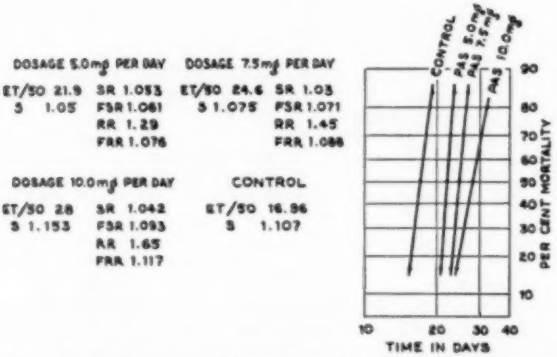

FIG. 4. Effect of para-aminosalicylic acid (PAS) administered subcutaneously.

the average values for control and treated groups. Figure 1 shows the time–per cent effect lines plotted from these average values, and it is evident from the fact that the RR exceeds the f_{RR} that 0.1 per cent pyrazinamide in the feed gave a significant prolongation of survival time. A statistical analysis of these results is presented in table 2. A comparison of pyrazinamide, 0.15 per cent in the diet, with *p*-aminosalicylic acid (PAS) at 0.2, 0.5, 0.75, and 1.0 per cent in the diet is shown in figure 2. The prolongation of survival time with 0.2 per cent of PAS in the diet is approximately the same as that for 0.1 per cent of pyrazinamide,

but the effect of 0.15 per cent of pyrazinamide is greater than that of 1 per cent of PAS. In figure 3 a group of infected mice treated with one subcutaneous dose of 2.5 mg. of pyrazinamide per day is compared with the average line for the nontreated control groups. The prolongation of survival time is significant.

The effects of subcutaneous injections of PAS and streptomycin are shown in figures 4 and 5. One subcutaneous injection per day of 5 mg. of PAS was required to equal 2.5 mg. of pyrazinamide per day subcutaneously. Doses of 7.5 and 10 mg. per day gave increased prolongation of survival time. Streptomycin, on the other hand, gave slightly better protection at a dose of 0.5 mg. subcutaneously once daily than did 2.5 mg. of pyrazinamide. One milligram of streptomycin per day gave markedly better results than 2.5 mg. of pyrazinamide per day.

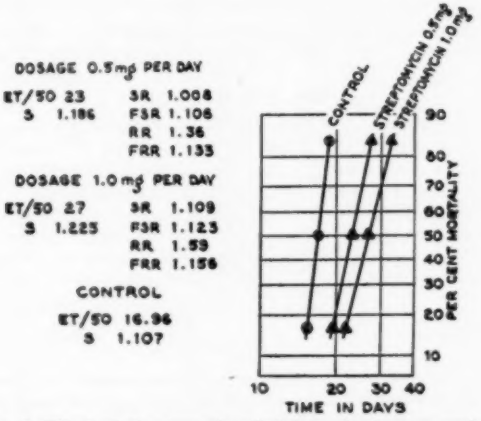

FIG. 5. Effect of streptomycin administered subcutaneously.

DISCUSSION

From the above results it would seem that the antituberculous activity of pyrazinamide (Aldinamide) in mice lies between that of streptomycin and that of PAS. Similar results have been observed in guinea pigs (5). In mice, a diet containing 0.1 per cent of pyrazinamide gave a prolongation of survival time roughly equivalent to that of 0.2 per cent of PAS, while 0.15 per cent of pyrazinamide in the diet gave results superior to those given by 1.0 per cent of PAS. When administered subcutaneously, a dose of 2.5 mg. of pyrazinamide per mouse per day was required to equal 0.5 mg. of streptomycin per day, but the pyrazinamide dosage was approximately equivalent to 5 mg. of PAS per mouse per day.

It might be helpful in further evaluating pyrazinamide to compare briefly the results obtained with PAS and streptomycin in the present studies with those from other laboratories. In table 3 is given the median survival time, in days, of mice given various dosages of PAS or streptomycin in each of four

laboratories. The tests of Baker, Schlosser, and White (6), Rake and associates (7), Youmans and Youmans (8), and that employed in the laboratory of the present writers are fundamentally similar in that they employ intravenously infected mice. The results differ in that a different strain of mice is employed in each laboratory. The first two groups mentioned use a bovine strain of tubercle

TABLE 3

CHEMOTHERAPEUTIC EFFECT OF PARA-AMINOSALICYLIC ACID (PAS) AND STREPTOMYCIN IN VARIOUS MOUSE TESTS

DRUG	DOSAGE		MEDIAN SURVIVAL TIME, DAYS			
	mg/kg	mM/kg	Baker *et al.* (6)	Rake *et al.* (7)	Malone *et al.*	Youmans and Youmans (8)
4-Aminosalicylic acid	270	1.76	15.0			
	400	2.64			21.2	
		2.77		22.9		
	530	3.46				25.0
	550	3.59	15.0			
	1,000	6.54		25.7	23.2	29.5
	1,200	7.84	17.0			
	1,500	9.80			26.8	
		10.08		26.8		
		12.77		28.1		
	1,900	12.4				44.0
	2,000	13.07			29.0	
	2,800	18.28	21.0			
Median survival time of non-treated mice			14.0	19.86	16.96	13.5
Streptomycin		0.0091		26.8		
	18.75	0.025				21.0
		0.026		30.2		
	25.0	0.034	15.0		23.0	
		0.039		32.5		
	37.5	0.05				45.0
	50.0	0.067	23.0		27.0	
	75.0	0.1				60.0
Median survival time of non-treated mice			12.0	19.86	16.96	12.5

bacillus, while Youmans and the present writers use the human H37Rv strain of tubercle bacillus; a different method of preparing inoculum is employed in each laboratory. It is not surprising, therefore, that considerable differences exist in the results obtained in different laboratories. In general, it appears that the test described by Baker, Schlosser, and White is the most severe and shows the least response to either drug. The test described by Rake and co-workers gave results surprisingly similar to those obtained in this laboratory when PAS

was the therapeutic agent employed, but the test of Rake and his associates showed greater sensitivity to streptomycin. With Youmans' test, a greater response to both drugs was shown than was true with any of the other three tests.

SUMMARY

A new therapeutic compound, pyrazinamide (Aldinamide) has been employed in a test for chemotherapeutic activity against tuberculosis in intravenously infected mice. A concentration of 0.1 per cent in the diet or 2.5 mg. per mouse per day administered subcutaneously produced a significant prolongation of survival time when measured by the time–per cent effect method of Litchfield.

A comparison has been made of the results obtained in this laboratory with PAS and streptomycin in mice with the results obtained in other laboratories with the same drugs in order to give a better basis for judgment of the tuberculostatic activity of pyrazinamide.

SUMARIO

El Efecto de la Aldinamida sobre la Tuberculosis Experimental en el Ratón

Un nuevo compuesto terapéutico, la pirazinamida (Aldinamida) ha sido empleado en un ensayo de su actividad quimioterapéutica contra la tuberculosis en ratones infectados endovenosamente. Una concentración de 0.1 por ciento en el alimento o de 2.5 mg. diarios por ratón administrada subcutáneamente fué seguida de una prolongación significativa del tiempo de sobrevivencia, medido por la técnica de efecto-tiempo-por ciento de Litchfield.

A fin de facilitar mejor base para juzgar la actividad tuberculostática de la Aldinamida, compáranse los resultados obtenidos con el PAS y la estreptomicina en el laboratorio de los AA. y en otros laboratorios.

RESUME

L'action de l'Aldinamide sur la tuberculose expérimentale de la souris

Un nouveau composé thérapeutique, la pyrazinamide (Aldinamide), a été employé au cours d'épreuves destinées à déterminer sou activité chimiothérapeutique contre la tuberculose de la souris, inoculée par voie intraveineuse. Le temps de survie, compté en temps-pour cent par la méthode de Litchfield, est prolongé de façon significative par l'administration sous-cutanée de 2,5 mg. par souris, par jour, ou par l'adjonction d'une concentration de 0,1% à la ration alimentaire.

Les résultats obtenus dans ce laboratoire avec le PAS et la streptomycine sont comparés avec les résultats fournis par les autres laboratoires pour ces mêmes produits, de manière à obtenir une base meilleure pour l'évaluation de l'activité tuberculostatique de l'Aldinamide.

Acknowledgment

The writers wish to express their thanks to Dr. Frank Wilcoxon and Mr. D. H. Reichard for advice and assistance with the statistical analysis.

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PYRAZINAMIDE (ALDINAMIDE[1]) IN EXPERIMENTAL TUBERCULOSIS OF THE GUINEA PIG[2]

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INTRODUCTION

Since pyrazinamide (Aldinamide) has been made available for biologic testing (1, 2), the compound has been given to approximately 900 guinea pigs infected with human strains of *M. tuberculosis* in the routine test for antituberculous activity used in this laboratory. The drug has also been given in the routine test to approximately 170 guinea pigs infected with bovine strains of *M. tuberculosis* and to approximately the same number of guinea pigs infected with human strains of *M. tuberculosis* for survival tests.

METHODS OF TESTING

The method of testing (3) is based on evaluation of the extent of the disease on postmortem examination. The estimated extent of disease in drug-treated animals is compared with that of a group of untreated controls and with that of a group treated with 4,4′-diaminodiphenylsulfone in the diet as a standard of reference. The disease is induced by intraperitoneal inoculation of a virulent strain of *M. tuberculosis* var. *hominis*. Drug treatment, started two weeks after inoculation, is continued for a period of three weeks.

OBSERVATIONS

Aldinamide as a tuberculostatic agent: The comparison of pyrazinamide (Aldinamide) with other antituberculous agents (table 1) is based on the average result of all of the observations. Activity of the drug is expressed in terms of a tuberculostatic guinea pig unit defined as "the minimal dose of any substance which has a tuberculostatic effect equal to that of the 4,4′-diaminodiphenylsulfone standard under the conditions of the test."

From the data presented in table 1 it appears that pyrazinamide (Aldinamide) given to the guinea pig in the diet is slightly less effective than 4,4′-diaminodiphenylsulfone; is more effective than *p*-aminosalicylic acid and nicotinamide; and is less effective than streptomycin given by the subcutaneous route.

Forms of administration: In the guinea pig, pyrazinamide (Aldinamide) is active after both oral and parenteral administration. The use of oily solutions and solution in propylene glycol did not prove more effective than the parenteral administration of aqueous solutions. By the oral route, the drug-diet method gave results inferior to those with forced feeding. There is a need for further investigation of ways of administration and of treatment schedules.

[1] Proprietary name, American Cyanamid Company.

[2] From Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York; and Summit Park Sanatorium, Pomona, New York.

Effect on different strains of tubercle bacilli: Most of the studies in guinea pigs were done with the same strain of human-type tubercle bacilli (S.P. #4314). Guinea pig experiments with a streptomycin-resistant strain (S.P. #2495) showed a response to pyrazinamide which was at least equal to that of strain S.P. #4313. The Washington Type Culture Collection's Bovine Strain #9805 of *M. tuberculosis* showed a rather poor response to pyrazinamide in the guinea pig.

Effect of combination with streptomycin and para-aminosalicylic acid (PAS): Combination of pyrazinamide with either streptomycin or PAS in the guinea pigs resulted in an additive antituberculous effect.

Effect of dosage: Establishment of a dose response curve for pyrazinamide was made difficult by very considerable variation of response to the same dosage with different samples of the drug. This variation was much more marked than in the case of both 4,4′-diaminodiphenylsulfone and streptomycin. Its cause has not yet been determined. Despite this variation in response, there is a general

TABLE 1

TUBERCULOSTATIC GUINEA PIG UNIT OF DIFFERENT SUBSTANCES WITH ANTITUBERCULOUS ACTIVITY

SUBSTANCE	FORM OF ADMINISTRATION			
	In the diet	Subcutaneously	Intraperitoneally	Orally
Streptomycin	—	15 mg.	—	—
4,4′-diaminodiphenylsulfone	50 mg.	—	—	50 mg.
PAS	300 mg.	—	—	—
Nicotinamide	200 mg.	200 mg.	—	200 mg.
Pyrazinamide	75 mg.	30–45 mg.	50 mg.	50 mg.

correlation between dosage and response both on oral and parenteral administration.

Lack of toxicity: Toxic effects were absent in guinea pigs which received 10 to 300 mg. of pyrazinamide daily (30 to 900 mg. per kg.) as judged by appearance and body weight during life and by post-mortem studies (gross and microscopic).

Pathologic studies: Preliminary histologic studies of the tuberculous lesions of pyrazinamide-treated guinea pigs failed to reveal a constancy of qualitative differences. In figure 1 the definite character of the quantitative differences may be seen. It would seem that all changes observed histologically could be explained by a tuberculostatic effect which resulted in a lowering of the actual infecting dose without essentially modifying the character of the disease. This would not necessarily apply to other animal species with a less progressive type of tuberculous disease.

Survival experiments: Two survival experiments with pyrazinamide (Aldinamide) had varying results. The first experiment was done with material which had been of low potency in the routine test. Survival rates in the treated animals did not differ significantly from those of the untreated guinea pigs. In a second experiment, the T_{50} differed significantly among three groups of 30 guinea pigs

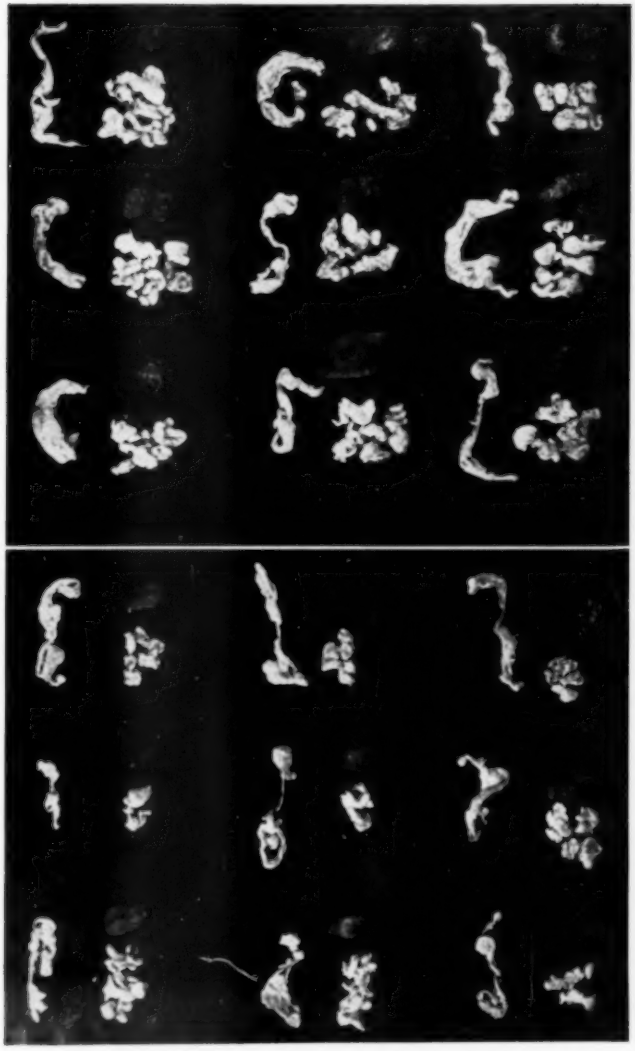

FIG. 1. Spleen, omentum, and lymph nodes of
(Upper) Nine untreated control guinea pigs
(Lower) Nine guinea pigs treated for three weeks with pyrazinamide

each: In *Group I* (controls) the T_{50} was 62 days; in *Group II* (30 mg. of drug daily subcutaneously for eight weeks) the T_{50} was 82 days; in *Group III* (75 mg. of drug daily in the diet for eight weeks) the T_{50} was 50 to 87 days.

SUMMARY

Pyrazinamide (Aldinamide) was tested against experimental tuberculosis in the guinea pig and was found to have moderate but definite antituberculous activity. The drug was active when administered by either the oral or the parenteral route and produced no evidences of toxicity in the dosages used.

SUMARIO

La Pirazinamida (Aldinamida) en la Tuberculosis Experimental del Cobayo

La pirazinamida (Aldinamida) fué ensayada contra la tuberculosis experimental en el cobayo y mostró actividad antituberculosa moderada, pero bien definida. La droga resultó activa al ser administrada por vía ya oral o parentérica sin evocar signos de toxicidad a las dosis utilizadas.

RESUME

La pyrazinamide (Aldinamide) dans le traitement de la tuberculose expérimentale du cobaye

La pyrazinamide (Aldinamide) a été expérimentée dans la tuberculose expérimentale du cobaye elle a fait preuve d'une action antituberculeuse modérée mais définie. Ce composé s'est montré actif par voie orale ou parentérale et il n'a pas été observé de signes de toxicité avec les doses utilisées.

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PYRAZINAMIDE (ALDINAMIDE[1]) IN THE TREATMENT OF PULMONARY TUBERCULOSIS[2,3,4]

R. L. YEAGER, W. G. C. MUNROE, AND FREDERICK I. DESSAU

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INTRODUCTION

In a program for the development of synthetic compounds with possible antituberculous activity at Lederle Laboratories by Kushner and associates (1), pyrazinamide (Aldinamide) was synthesized.

Following *in vitro* studies with pyrazinamide, Malone and co-workers (2) demonstrated that in experimental tuberculosis in mice there was a significant prolongation of survival time in pyrazinamide-treated animals and that lung involvement in the treated mice was considerably less than in the untreated control animals.

Studies in experimental tuberculosis in guinea pigs made by Dessau and co-workers (3), using a test method based on evaluation of the extent of disease (4), showed that, when pyrazinamide (Aldinamide) was given in the diet, it was slightly less effective than 4,4′-diaminodiphenylsulfone, but was more effective than *p*-aminosalicylic acid (PAS) and nicotinamide. Pyrazinamide was less effective than streptomycin when given by the subcutaneous route. Toxic effects were absent in the guinea pig studies.

As a consequence of these findings, an investigation was started at the Summit Park Sanatorium to observe the effect of pyrazinamide in the treatment of pulmonary tuberculosis in man. Treatment was started on the first patient on September 16, 1949. The present report which represents 43 cases presents the salient observations in all patients treated. Treatment was started on the last patient in the series on December 8, 1951.

PLAN OF INVESTIGATION

Choice of Patients

When pyrazinamide was first made available, nothing was known concerning its toxicity in humans. As a result, the initial studies were made with great caution. On the whole, patients were chosen who had had far advanced tuberculosis for many years and had failed to respond to adequate hospitalization, operative procedures, or other treatment. In most instances, tubercle bacilli recovered from this group of patients were resistant to streptomycin *in vitro*, and the patients' infections had been proved resistant by failure to respond to adequate doses of the drug.

At first, patients were chosen who showed symptoms of fever, cough, and expectoration

[1] Proprietary name, American Cyanamid Company.

[2] From Summit Park Sanatorium, Pomona, New York.

[3] Presented at the Eleventh VA-Army-Navy Conference on the Chemotherapy of Tuberculosis, St. Louis, Missouri, January 19, 1952.

[4] This study was supported jointly by the Lederle Laboratories Division, American Cyanamid Company, and the Summit Park Research Fund.

TABLE 1

	NAME	DATE STARTED	TOTAL GRAMS	TOTAL DAYS	ROENTGENOGRAMS		CLINICAL		TOXICITY	REMARKS
					Before	After	Temperatures	Other		
1.	FS	9/16/49	31	10	MA with cavity	Same	x	0	0	Treatment stopped due to streaking; patient left against advice.
2a.	JD	9/16/49	174	28	FA with cavity	Same	xxxx	x	Joint pains	Marked symptomatic improvement during treatment; symptoms recurred three weeks later.
2b.	JD	11/17/49	190	36	FA with cavity	Same	xxx	No change	Joint pains	Less response; died one year later despite antimicrobials.
3.	HH	9/16/49	3.5	3	FA with cavity	Same	0	No change	Abdominal discomfort	Uncooperative patient.
4.	JC	10/5/49	251	33	FA with cavity	Same	xxx	No change	Joint pains	Symptomatic improvement slowly lost; still active despite antimicrobials.
5.	VN	10/26/49	329	55	FA with cavity	Same	x	xx	Joint pains	Symptomatic improvement slowly lost; still active despite antimicrobials.
6.	FdeG	10/26/49	114	24	FA with cavity	Same	xx	No change	Joint pains	Immediate symptomatic improvement but return of symptoms during treatment.
7a.	MJ	11/7/49	342	48	FA with cavity	Same	xxx	x	Joint pains	Marked symptomatic improvement; pleurocutaneous sinus closed.
7b.	MJ	2/9 /50	303	60	FA with cavity	Same	xx	x	Joint pains	Less improvement.
7c.	MJ	6/10/50	50	15	FA with cavity	Same	x	No change	Joint pains	No response.
8a.	VM	1/11/50	492	75	FA with cavity	Same	xxxx	xx	Joint pains	Marked symptomatic improvement; slow return.

8b.	VM	4/12/50	43	13	FA with cavity	Same	0	No change	None	No response; death later.
9.	JF	1/19/50	30	6	FA without cavity	Same	0	No change	None	Fever due to periarteritis; no response.
10.	RF	2/21/50	427	70	MA with cavity	Improvement	xxx	xxx	0	Continued improvement with no further antimicrobials or collapse therapy; discharged inactive.
11a.	HS	3/14/50	40	8	FA without cavity	Same	Normal	No change	0	Tuberculous ulcer, base tongue, rapid clearing.
11b.	HS	12/9/50	186	73	FA without cavity	Same	Normal	No change	0	Tuberculous abscess, sternum, disappeared; sputum negative since treatment.
12.	CP	3/24/50	173	52	MA with cavity	Improvement	Normal	x	Palpitation	Marked roentgenographic and clinical response but cavity returned after treatment; later improvement with streptomycin and pneumothorax.
13.	LB	3/25/50	114	51	FA with cavity	Same	Normal	xx	Joint pains	Slight improvement slowly lost; still active despite further antimicrobials.
14.	LI	4/1/50	169	31	FA with cavity	Same	xxxx	xx	Joint pains	Marked improvement slowly lost after treatment; death despite further antimicrobials.
15.	GF	4/3/50	235	43	MA with cavity	Improvement	xxx	xxx	None	Marked improvement; discharged inactive without further antimicrobials or collapse therapy.
16.	OC	4/11/50	146	38	Negative	Same	Normal	Negative	None	Negative chest; axillary adenitis improved slightly; later treated with streptomycin.

TABLE 1—*Continued*

	NAME	DATE STARTED	TOTAL GRAMS	TOTAL DAYS	ROENTGENOGRAMS		CLINICAL		TOXICITY	REMARKS
					Before	After	Temperatures	Other		
17.	LB	4/25/50	144	37	FA without cavity	Same	x	xx	None	Slight improvement; continues active, good chronic.
18.	GB	5/3/50	55	19	FA with cavity	Same	Increase	No change	Febrile	Subsequent allergic reaction to streptomycin; arrested after thoracoplasty.
19.	IF	6/13/50	43	13	FA with cavity	Same	Increase	No change	Febrile	Continued slow activity.
20.	EF	6/13/50	127	31	FA with cavity	Same	Normal	x	Joint pains	Slowly progressive despite antimicrobials.
21.	AR	6/19/50	128	41	MA without cavity	Same	No change	Negative	None	Treated for tuberculous empyema; no response; later decortication and thoracoplasty.
22.	FG	7/3/50	231	58	FA with cavity	Same	xxx	No change	None	Subsequent death despite antimicrobials.
23.	JH	7/8/50	90	22	FA with cavity	Same	Increase	xxxx	Slow febrile	Slowly increasing fever during treatment; later response to streptomycin and pneumoperitoneum.
24.	JH	7/7/50	146	51	FA with cavity	Same	xxxx	xx	None	Acute spread improved; returned to good chronic phase.
25.	NT	7/18/50	148	43	FA with cavity	Improvement	xxxx	No change	Joint pains	Acute spread improved; returned to slowly progressive chronic; no response to later course or other antimicrobials; still active.
26.	IZ	8/7/50	145	51	MA without cavity	Improvement	Normal	x	Palpitation	Roentgenographic clearing, but small cavity remained after treatment requiring collapse.

27.	AH	8/14/50	64 (interrupted)	37	MA without cavity	Improvement	Normal	Negative	Acute febrile	Treatment interrupted but resumed with no further toxicity; discharged inactive with no further antimicrobials or collapse.
28.	FC	9/9/50	181	47	FA with cavity	Slight Improvement	Normal	x	None	More marked improvement with streptomycin and thoracoplasty.
29.	FB	10/26/50	154	57	MA without cavity	Slight Improvement	Normal	Negative	None	Continued improvement; no further antimicrobials or collapse; discharged inactive.
30.	RK	11/6/50	35.8	14	MA without cavity	Same	x	No change	Febrile	Treatment stopped due to chills; subsequent slow improvement with other antimicrobials.
31.	EL	11/21/50	73	22	FA with cavity	Spontaneous Pneumothorax	xxx	xxx	None	Development of bronchopleural fistula; still active despite antimicrobials.
32a.	CK	12/6/50	53	14	FA with cavity	Improvement	Normal	xx	0	Treatment interrupted at request of patient.
32b.	CK	1/23/51	164	73	FA with cavity	Improvement	Normal	xxx	Jaundice	Marked clinical and roentgenographic improvement; developed jaundice subsequent clearing.
33.	CJ	12/9/50	106	41	MA with cavity	Improvement	xxx	?	None	Left against advice during treatment.
34.	AB	1/21/51	16	7	FA with cavity	Same	Normal	—	Difficulty raising	Treatment stopped at request of patient; no change.
35.	JS	1/20/51	102	37	FA without cavity	Improvement	xxxx	x	None	Continued rapid improvement with pneumoperitoneum; no further antimicrobials.
36.	RH	3/26/51	22	9	MA without cavity	Same	Normal	xx	Nervousness, palpitation	Treatment stopped at request of patient.

TABLE 1—*Concluded*

	NAME	DATE STARTED	TOTAL GRAMS	TOTAL DAYS	ROENTGENOGRAMS		CLINICAL		TOXICITY	REMARKS
					Before	After	Temperatures	Other		
37.	AH	4/23/51	142	53	MA with cavity (with siderosis)	Same	Normal	No change	None	Siderotuberculosis; no change; subsequent resection.
38.	CP	4/23/51	142	52	MA with cavity	Improvement	xxx	xxx	Jaundice	Treatment stopped due to jaundice; subsequent clearing; patient still active.
39.	JO'M	5/10/51	168	72	FA with cavity	Improvement	x	xx	None	Marked clinical improvement with rapid clearing with tuberculous laryngitis; left against advice.
40.	LC	8/25/51	80	29	MA with cavity	Improvement	0	No change	0	Marked improvement; roentgenographic relapse after treatment stopped; slow improvement with other antimicrobials; still active.
41.	AA	9/24/51	24	12	FA with cavity	Same	xx	Slight improvement	0	Some improvement *chronic* tuberculous laryngitis; further improvement when changed to streptomycin.
42.	EF	12/1/51	32	13	FA with cavity	Same	Slight improvement	Slight improvement	0	Some improvement *chronic* tuberculous laryngitis; further improvement when changed to streptomycin.
43.	MG	12/8/51	113	42	MA with cavity	Improvement	xxxx	xxxx	0	Marked clinical and roentgenographic improvement with immediate temperature drop.

M.A. = moderately advanced
F.A. = far advanced

due to active tuberculosis. Their films showed a far advanced fibrotic cavitary type of tuberculosis which had been slowly progressive despite other treatment.

Subsequently, patients with less advanced disease were studied who had had no previous treatment with antimicrobial therapy.

Regimen

Pyrazinamide[5] (Aldinamide) was given in capsules immediately after meals or with fluids. The dosage schedule varied considerably from patient to patient. The largest sustained dose based on weight of the patient was 0.2 gm. per kg. per day. The largest sustained total daily dose was 8.4 gm. for a thirty-three-day period. One patient received a total of 695 gm. administered in three separate courses. The final treatment schedule selected consisted of 350 mg. administered four times the first day and 700 mg. four times per day thereafter for forty-two days.

With the original higher dosage there were some complaints voiced, principally of bitter after-taste or abdominal discomfort. The latter was easily relieved by antacids. With the final dosage schedule complaints were few.

Determination of Drug Resistance in Vitro

Tubercle bacilli isolated from untreated cases were studied for susceptibility to pyrazinamide *in vitro*. A modification of the standard streptomycin susceptibility method was used. A fairly large inoculum was used in tubes containing 0, 1, 10, 100, and 1,000 γ of pyrazinamide per ml., and the growth of the culture was measured by a turbidimetric method (5). Complete inhibition of growth was seen at concentrations of 100 and 1,000 γ of pyrazinamide per ml. Partial inhibition was noted at 10 γ and occasionally at 1 γ per ml.

RESULTS

Toxicity

Toxic reactions on the whole were mild and are presented in detail in table 1.

The hazard in using a new experimental compound in a small institution can readily be appreciated. Any change in the patient's status, particularly any detrimental change, was at once ascribed to the medication. Thus, when a patient under treatment complained of severe joint pains, subsequent patients watched for this symptom with apprehension.

Joint pains: Ten patients complained of joint pains. The joints usually involved were the shoulders, elbows, or knees. Movement of the joint was limited by pain. No redness, swelling, or tenderness was apparent. No changes were found on roentgenographic examination. In 3 cases, the pain was of such severity that medication was discontinued. In all patients the symptoms slowly disappeared after termination of treatment. Moreover, joint pains were not complained of at all by the patients on the final lower dosage regimen.

Four patients had nervousness, palpitation, or a feeling of tightness in the chest. After brief respite they were able to continue without the recurrence of symptoms.

Febrile reaction: Three patients had an acute febrile reaction during treatment, with chills and temperature to 103°F. which fell to normal within twenty-four hours. An illustration of this type of reaction may be seen in figure 12 (Case A. H.). In all of the 3 patients the medication was immediately discon-

[5] In the pure chemical form.

tinued, but in one it was subsequently resumed without giving rise to any symptoms.

Eosinophilia: A few patients had eosinophilia ranging from 5 to 10 per cent. No anemia or agranulocytosis was observed.

Jaundice: Two patients became jaundiced during treatment, one after 142 gm. of pyrazinamide had been given for a period of forty-two days; the other, after 159 gm. in a period of eighty-seven days. In both patients there was a preliminary period of anorexia and general lassitude followed by evident jaundice. Medication was immediately stopped and the patients were maintained on a high carbohydrate, high protein diet with ample fluids. Nausea was present. The jaundice reached a peak after a few days, then slowly receded. Tests of liver function suggested parenchymal involvement, which slowly cleared. At the present time, six and eight months after termination of treatment, these 2 patients have no residual signs of hepatic disease.

Twenty-one patients had no complaints of any kind attributable to pyrazinamide administration.

TOXIC SYMPTOMS OBSERVED IN 43 PATIENTS WHO RECEIVED PYRAZINAMIDE (ALDINAMIDE)

SYMPTOMS	NUMBER OF PATIENTS
Joint pains	10
Eosinophilia 4 to 10$\frac{1}{4}$	10
Palpitation	4
Jaundice	2
Chills, fever	3
Slowly rising fever	1
Difficulty in raising	2
Pigmentation	2
None	21

Antituberculous Activity

Defervescence: Within one to three days after the start of pyrazinamide administration, all but one of the tuberculous patients who had fever of more than 1°F. showed a complete defervescence. The remission of fever was also accompanied by improvement in subjective feeling characterized by improved appetite and a general feeling of well-being that occasionally was euphoric. This was followed by diminished cough and reduction in the total amount of sputum.

Representative examples of the defervescence observed may be seen in figures 1, (Case J. D.), 4 (Case V. M.), and 3 (Case M. J.).

Roentgenographic changes: Little roentgenographic change was expected in the patients who were initially treated, as their films showed mainly a far advanced fibrotic cavitary type of disease which had slowly progressed despite various forms of collapse and antimicrobial therapies. Twenty-five patients had far advanced disease. In 19 there were no significant roentgenographic changes at the end of the treatment period. In 6 of these patients, however, there was a definite improvement in the roentgenographic shadows of more recent disease.

This was noted both when the greater part of the disease was of a recent exudative nature and when fresh areas of disease were superimposed on an old far advanced tuberculous process. Examples of defervescence and roentgenographic findings at the onset of treatment may be seen in figures 9 (Case J. S.), 8 (Case N. T.), and 7 (Case J. H.).

Fifteen patients had moderately advanced active disease. Of these, 12 showed roentgenographic improvement, and in 3 the shadows on the films remained unchanged. Representative examples of the roentgenographic findings in these patients with moderately advanced tuberculosis may be seen in figures 5 (Case R. F.), 6 (Case G. F.), and 10 (Case M. G.).

Extrapulmonary lesions: Two patients had an acute tuberculous laryngitis showing edema, redness, swelling, surface ulceration, and pain on swallowing. After the start of pyrazinamide therapy, the lesions cleared rapidly with early loss of pain and other signs. In 2 other patients, chronic laryngeal lesions which had been present and slowly progressive for a period of several years showed only slight improvement on pyrazinamide therapy. Both lesions responded more rapidly when pyrazinamide was discontinued and streptomycin was started.

One patient had a small indurated ulcer at the base of the tongue which was proved to be of tuberculous origin by examination of biopsy material. The ulcer healed rapidly and remained well after seven days' treatment with pyrazinamide. The patient subsequently developed a cold abscess to the left of the sternum which cleared slowly after seventy-three days of pyrazinamide treatment, during which time his advanced pulmonary disease also improved.

In another patient, a small draining pleurocutaneous fistula communicating with a poorly drained empyema cavity closed under pyrazinamide treatment. The fistula had previously been unaffected by several courses of streptomycin. The underlying empyema cavity and larger posterior drainage tract were not noticeably affected by the pyrazinamide.

A patient with active tuberculous adenitis in the left axilla showed marked reduction in some of the nodes during the administration of pyrazinamide. During therapy, however, other nodes became palpable along the rib and were subsequently resected during a course of streptomycin therapy.

One patient treated for tuberculous empyema with inactive pulmonary tuberculosis showed no symptomatic or other response to pyrazinamide.

Question of nonspecific antipyrexial effect: In an attempt to see if the rapid defervescence of the tuberculous patients represented a nonspecific antipyrexial effect, 2 patients with fever of other origin were treated with pyrazinamide. One of these patients had inactive tuberculosis but had definite periarteritis nodosa with a daily temperature as high as 104°F. There was no evidence of any influence of the drug on the febrile course of this patient (figure 11, Case J. F.). The other patient had fever as a result of a nontuberculous pleural effusion, and his temperature course was also unaffected by the administration of pyrazinamide.

Bacteriologic studies: Thirty-three patients were discharging tubercle bacilli in the sputum when pyrazinamide treatment was started. In 15 there was no

change in the bacteriologic findings during therapy. The bacillary content was reduced in 18 patients and, in 10 of the 18, tubercle bacilli could no longer be found on smear of a concentrated specimen. In 4 cases, the sputum remained negative for tubercle bacilli on all subsequent cultures without further drug therapy.

Emergence of bacterial resistance. There was a rapid and irregular emergence of drug-resistant strains of tubercle bacilli which was accompanied by failure to maintain symptomatic response, despite continued treatment, and failure to respond to a repeated course of treatment after relapse. An example of such a relapse on the fifteenth day of continued pyrazinamide therapy may be seen in figure 2 (Case F. deG.). Pyrazinamide-resistant tubercle bacilli seemed to appear more rapidly in those patients who had large cavities and large quantities of sputum with high bacillary content.

Development of an appreciable degree of drug resistance was noted in all of the 12 patients whose bacilli were tested before and after treatment. All of the strains isolated after therapy showed growth in a pyrazinamide concentration of 100 γ per ml., and strains from 10 patients grew in 1,000 γ of drug per ml.

Relapse: Because of the type and chronicity of the lesions in most of the patients treated, permanent improvement was neither anticipated nor seen in most cases. In the group of far advanced cases, relapse after treatment was common. Fourteen of 17 patients who were treated with pyrazinamide for thirty days or longer returned to their pretreatment phase one month to one year after treatment. In these 14, the tuberculosis has either proved fatal or remains active despite other antimicrobial therapy.

Of 10 patients with moderately advanced disease who were treated thirty days or longer, relapse or a return to their pretreatment phase was noted in four.

DISCUSSION

In reviewing the effect of pyrazinamide (Aldinamide) on the course of the tuberculosis in these patients, it appears that the drug possesses a moderate degree of antituberculous activity. Pyrazinamide was capable of producing a rapid defervescence in patients with fever due to active pulmonary tuberculosis, and the administration of the drug was uniformly associated with reduction in cough and total amount of sputum and with the appearance of a general subjective improvement. The effects of pyrazinamide were equally apparent in patients whose tubercle bacilli were resistant to streptomycin as in those whose bacilli were susceptible. The toxic reactions encountered were mild and, as a whole, unimportant.

The immediate symptomatic response, which sometimes reaches a euphoric level, is more rapid than that previously seen by the present writers following the use of streptomycin or PAS.

Unfortunately, the usefulness of pyrazinamide appears to be limited sharply because of the rapid emergence of drug-resistant strains of tubercle bacilli in the cases of far advanced tuberculosis. Relapse was common in this group and was also seen frequently in the cases of moderately advanced disease after the

short courses of drug used in the present study. Even in the last-named situation, however, the temporary improvement noted may be of real benefit. It is possible, moreover, that longer courses of treatment would be beneficial in the moderately advanced lesions. This aspect of the problem is under investigation.

In the present study, the use of pyrazinamide in conjunction with other antimicrobials has been avoided in an attempt to evaluate its effects alone. Pyrazinamide was used with PAS for short periods in 2 cases.

It is believed that pyrazinamide has a definite sphere of usefulness in the following situations in tuberculosis: (*1*) in patients with strains of tubercle bacilli which are resistant to streptomycin, who have recent increase of disease, and in whom treatment might enable them to return to a "good chronic" phase; (*2*) in patients with streptomycin-resistant bacilli who need surgical treatment; (*3*) in patients with moderately advanced or early far advanced active exudative disease with fever, cough, and expectoration.

SUMMARY

Pyrazinamide (Aldinamide) has been used in the treatment of 43 patients with active pulmonary tuberculosis. The administration of the drug was promptly followed by a rapid reduction of temperature in those patients with more than one degree of fever. The severity of cough and the total amount of sputum were considerably diminished. Pyrazinamide appeared to be equally effective in patients whose tubercle bacilli were resistant or susceptible to streptomycin. The toxic reactions noted during the administration of the drug were all mild. The usefulness of pyrazinamide is limited by the rapid emergence of drug-resistant bacilli, particularly in patients with far advanced tuberculosis with large cavities.

SUMARIO

La Pirazinamida (Aldinamida) en el Tratamiento de la Tuberculosis Pulmonar

La pirazinamida (Aldinamida) fué usada en el tratamiento de 43 enfermos con tuberculosis pulmonar activa. La administración de la droga fué pronto seguida de rápida baja de la temperatura en los enfermos que tenían más de 0.6 grados de fiebre. Disminuyeron considerablemente la intensidad de la tos y la cantidad total de esputo. La pirazinamida pareció ser igualmente eficaz en los enfermos cuyos bacilos tuberculosos eran resistentes o susceptibles a la estreptomicina. Todas las reacciones tóxicas observadas durante la administración de la droga fueron leves. La utilidad de la pirazinamida se ve limitada por la rápida formación de bacilos farmacorresistentes, en particular en los sujetos con tuberculosis muy avanzada y cavernas grandes.

RESUME

La pyrazinamide (Aldinamide) dans lé traitement de la tuberculose pulmonaire

La pyrazinamide (Aldinamide) a été utilisée dans le traitement de 43 malades atteints de tuberculose pulmonaire en activité.

L'administration de ces médicaments a été immédiatement suivie d'un abaissement rapide de la température chez ceux des malades dont la température dépassait de 1 degré la normale. La sévérité de la toux et la quantité totale de l'expectoration étaient considérablement diminuées. La pyrazinamide s'est montrée également efficace chez des malades dont les bacilles tuberculeux étaient résistants ou sensibles à la streptomycine. Les réactions

d'intolérance, observées au cours de l'administration de ce médicament, ont été toutes légères. L'utilité de la pyrazinamide est limitée par l'apparition rapide de bacilles résistants à son action, en particulier dans les cas de tuberculose très avancée avec cavernes importantes.

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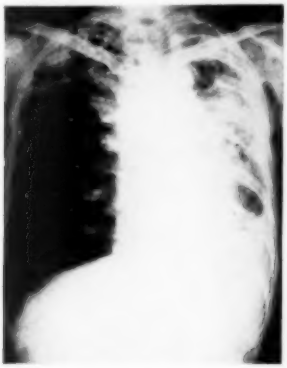
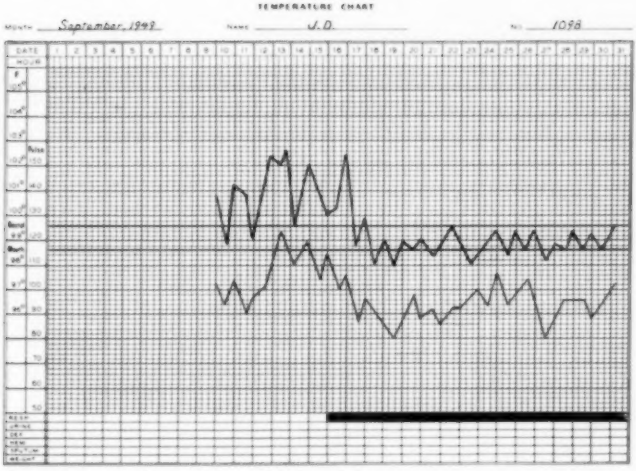


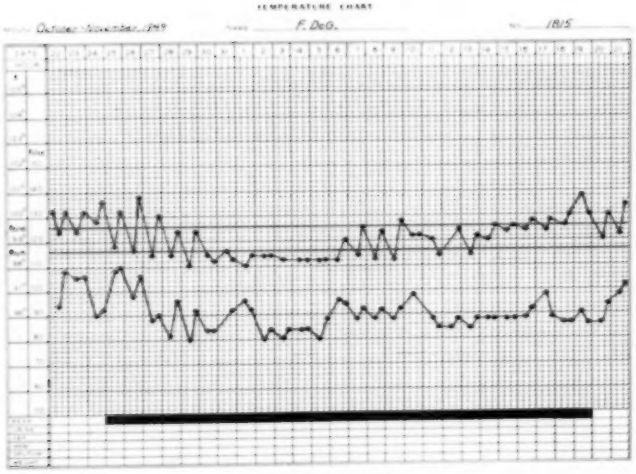


Case 2 (J. D.)

FIG. 1. Fifty-eight-year-old male. Onset of tuberculosis in 1926. Continuous hospitalization since 1936. Film at start of treatment showed: *right:* fibrotic infiltrate and cavity formation in the upper third; *left:* cavity 6 by 9 cm. in upper half, increasing disease and cavity formation below that area. The sputum contained tubercle bacilli, temperature ranged 101° to 103°F. for two months. He was previously treated with streptomycin on three occasions with no response in the last course, and tubercle bacilli recovered from sputum showed growth in 100 γ of streptomycin per ml. Treatment with pyrazinamide (Aldinamide), September 16, 1949 to October 13, 1949, 174 gm. There was an immediate drop of temperature to normal and sputum was reduced to one ounce. He relapsed within twenty-one days after treatment was stopped. A second course gave less response. A third course, one year later, gave no response. He died despite subsequent antimicrobials.

Roentgenogram taken September 16, 1949.

Temperature chart indicating commencement of pyrazinamide therapy.

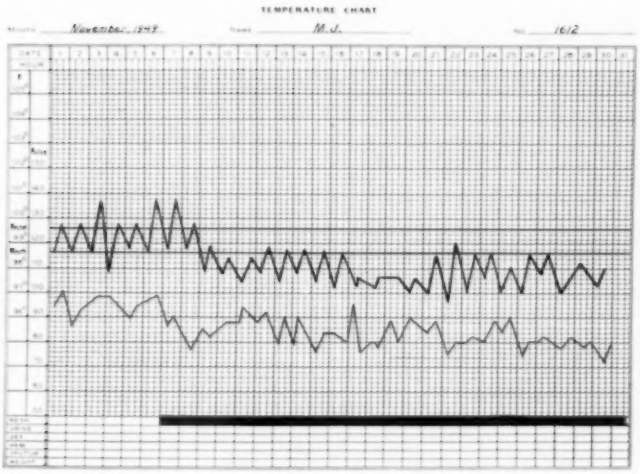


Case 6 (F. de G.)

FIG. 2. Thirty-two-year-old female with pulmonary tuberculosis treated since 1936 with right and left pneumothorax, left thoracoplasty. Her roentgenograms 1946 to 1949 showed a large cavity at both right and left apex. There was a spreading lesion in the right base with increasing cavity formation noted in 1949. Streptomycin was given for 84 days in 1948–1949, with subjective improvement but no roentgenographic change. Patient's temperature rose to 101°F. daily for one month at time treatment was started. The sputum was positive for tubercle bacilli. Bacilli grew in presence of 100 γ of streptomycin per ml. Total treatment, 114 gm. in twenty-four days. Her temperature immediately dropped to normal but returned to the previous level by the seventeenth day. The following year she was again tried on pyrazinamide with no response. She died January 1951 despite further antimicrobials. This case illustrated the rapid emergence of resistance both clinically and *in vitro*. The bacilli recovered from this patient before treatment showed little susceptibility. The appearance of highly resistant strains of bacilli occurred rapidly.

Roentgenogram taken October 7, 1949.

Temperature chart indicating commencement of pyrazinamide therapy.

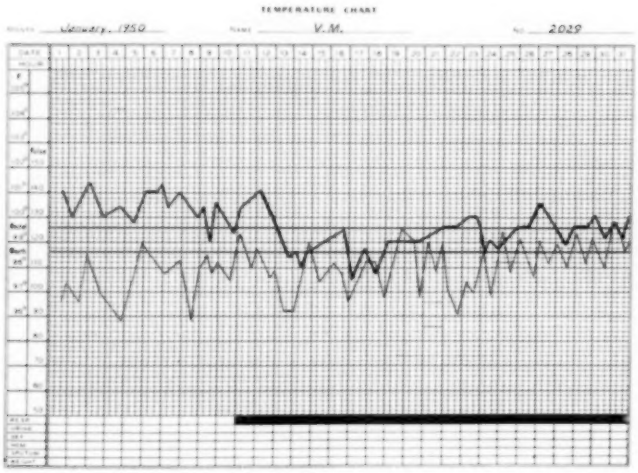


Case 7 (M. J.)

FIG. 3. Thirty-three-year-old female. Left pneumonectomy for tuberculosis in 1944. Tuberculous empyema and bronchopleural fistula one year later with spread throughout remaining lung. Temperature to 101°F. for more than two months. Previously treated with streptomycin. Pyrazinamide (Aldinamide) started on November 7, 1949 and continued for 48 days. Immediate symptomatic response. A draining anterior pleurocutaneous sinus closed; no change in draining empyema. Recurrence of fever at end of six weeks with less response to re-treatment. Still active despite all other antimicrobials.

Roentgenogram taken October 17, 1949.

Temperature chart indicating commencement of pyrazinamide therapy.

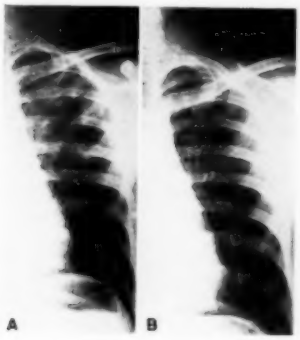


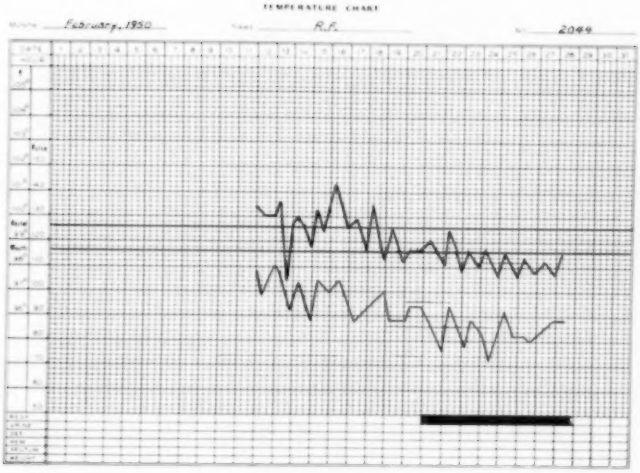
Case 8 (V. M.)

FIG. 4. Forty-nine-year-old male. Onset of tuberculosis in 1934, with thoracoplasty in 1936. "Well" until 1949, but with no check-up for several years. He had gradual development of symptoms culminating in an acute symptomatic flare-up in October 1949. Admitted in November 1949. His roentgenogram showed: *right:* thoracoplasty; *left:* infiltration throughout. Cavity formation in both upper thirds. He was treated with streptomycin which was discontinued due to slowly rising nonprotein nitrogen. His temperature rose to 101°F. Pyrazinamide was started on January 11, 1950, followed by a rapid drop in temperature and improved symptoms. Total drug, 536 gm. in 91 days, two courses. There was little or no response to second course. He died in August 1950.

Roentgenogram taken October 28, 1949.

Temperature chart indicating commencement of pyrazinamide therapy.



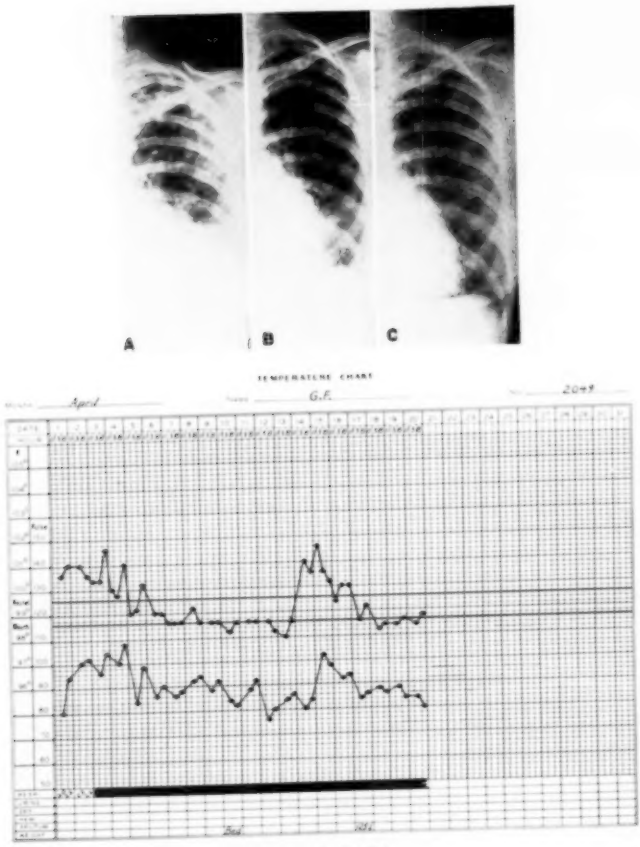


Case 19 (R. F.)

FIG. 5. Twenty-three-year-old male. A diagnosis of tuberculosis was made on routine insurance examination five days before admission. His temperature was elevated on admission, falling to normal before treatment with pyrazinamide was started. His roentgenogram showed a very active lesion in the left upper third which progressed following a small hemorrhage. Sputum was positive for tubercle bacilli. Treatment started on February 2, 1950 and continued for a total of 70 days, 427 gm. The sputum was negative for tubercle bacilli on concentrate and culture after treatment, with clearing of roentgenogram. No further antimicrobials or collapse therapy. Discharged as inactive May 1951.

A. Roentgenogram taken February 2, 1950 before commencement of pyrazinamide therapy.

B. Roentgenogram taken June 5, 1950. Temperature chart indicating commencement of pyrazinamide therapy.



Case 15 (G. F.)

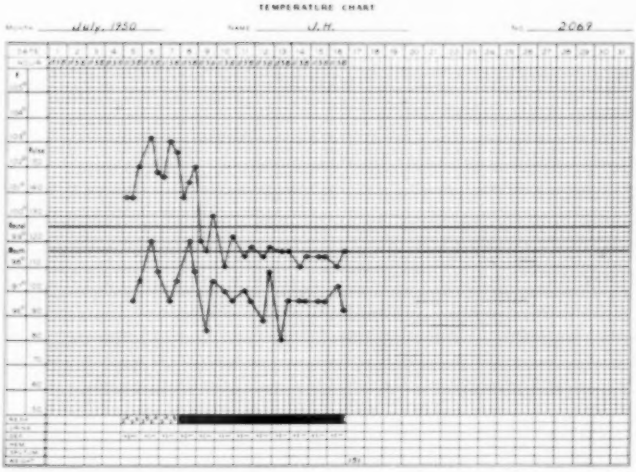
FIG. 6. Thirty-four-year-old female. Symptomatic onset of disease in March 1951; was admitted March 30, 1951 with temperature to 101°F. Her roentgenogram showed: *right:* nothing; *left:* an active infiltrate throughout with fluid at base. The sputum was positive for tubercle bacilli. Pyrazinamide was started on April 3, 1950 and continued for 45 days, 235 gm. The temperature fell to normal and remained so except for a transient rise. The sputum became negative. Roentgenograms showed marked clearing. No further antimicrobials or collapse therapy. Was discharged inactive in April 1951.

A. Roentgenogram taken March 28, 1950.

B. Roentgenogram taken June 16, 1950.

C. Roentgenogram taken March 1, 1951.

Temperature chart indicating commencement of pyrazinamide therapy.

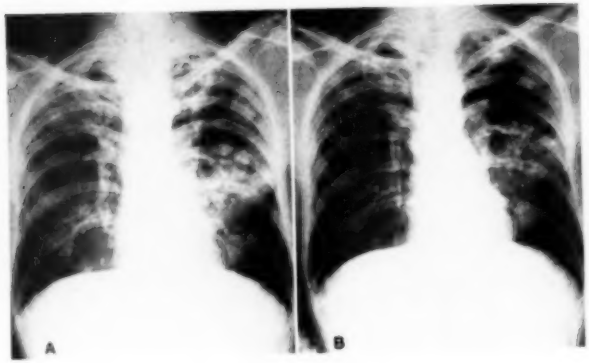


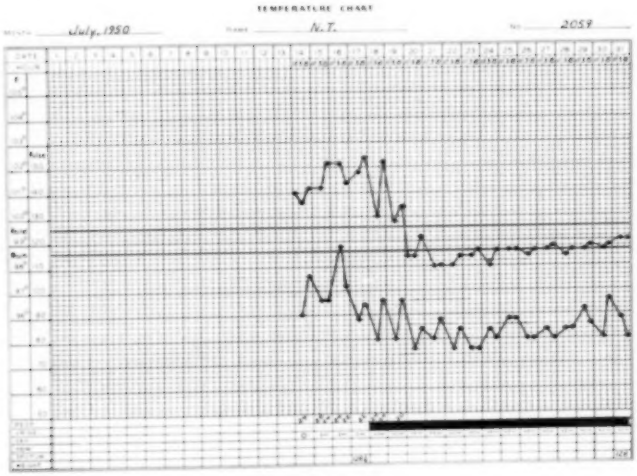
Case 24 (J. H.)

FIG. 7. Fifty-nine-year-old male. Far advanced tuberculosis diagnosed in 1945, with treatment since that time. Had sudden spread of disease left lower half, with temperature to 103°F., June 1950. No response to aureomycin or penicillin. Pyrazinamide was started on July 7, 1950 and continued for 51 days. Definite symptomatic response and roentgenographic clearing of recent lesion. Temperature remained normal for the next two months, at which time patient started on PAS due to continued cough. Still active despite streptomycin and PAS.

Roentgenogram taken July 6, 1950.

Temperature chart indicating commencement of pyrazinamide therapy.





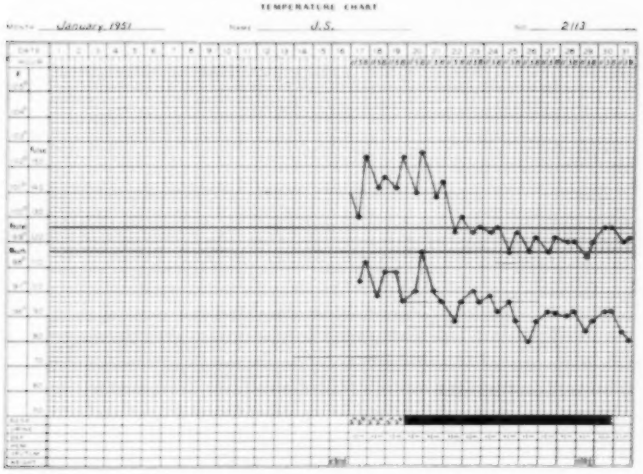
Case 25 (N. T.)

FIG. 8. The patient, a 47-year-old-male, was admitted June 5, 1950. He had previously been treated with streptomycin to the point of resistance, bacilli growing in presence of 1,000 γ of streptomycin per ml. His roentgenogram showed a far advanced lesion bilaterally with cavity formation in both upper one-thirds. The sputum was highly positive for tubercle bacilli. He developed an acute lesion in the left middle one-third, with increasing fever six weeks prior to pyrazinamide therapy. Treatment started on July 18, 1950 and continued for 43 days; total drug 148 gm. There was marked immediate symptomatic response and clearing of roentgenogram. He subsequently relapsed and gave no response on second treatment. He also showed no response to subsequent antimicrobials. He is still active.

A. Roentgenogram taken July 17, 1950.

B. Roentgenogram taken September 6, 1950.

Temperature chart indicating commencement of pyrazinamide therapy.

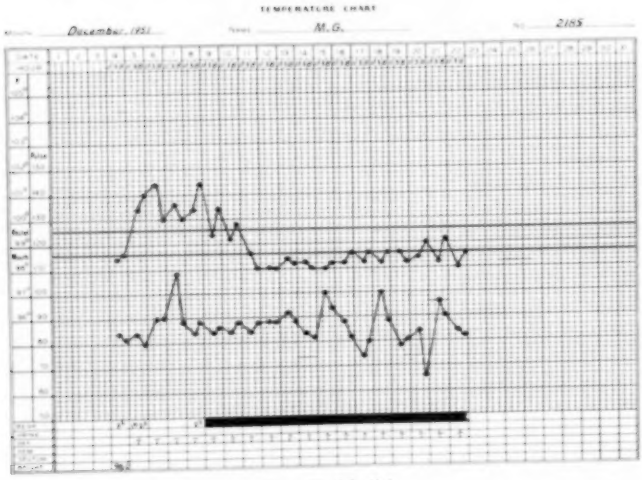


Case 35 (J. S.)

FIG. 9. The patient, a 49-year-old male was admitted January 12, 1951, one week after having broken a rib. He was acutely ill. His roentgenograms showed: *right:* some infiltration at the base; *left:* infiltration of an active nature throughout. The temperature rose to 102°F. daily. Sputum contained 100 to 200 cc. of acid-fast bacilli. He was treated three days with PAS without response. This was stopped and pyrazinamide started on January 20, 1951; total amount 101 gm. in 37 days. He had rapid symptomatic and roentgenographic response and subsequent further clearing with pneumoperitoneum. No further antimicrobials.

Roentgenogram taken January 20, 1951.

Temperature chart indicating commencement of pyrazinamide therapy.



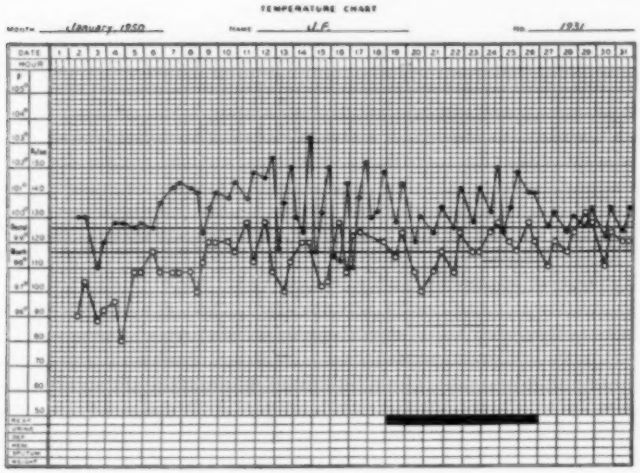
Case 43 (M. G.)

FIG. 10. Thirty-one-year-old female with symptomatic onset of tuberculosis in September 1951. A diagnosis of pneumonia was made November 22, 1951, at which time her temperature was to 102°F. There was no response to penicillin, aureomycin, or terramycin. The sputum was found positive for tubercle bacilli, and she was transferred to the Sanatorium with temperature remaining at 102°F. daily. The roentgenograms showed a very active lesion with infiltration on the right middle third and base: *left:* scattered infiltrate throughout with consolidation at base. The sputum contained large numbers of acid-fast bacilli. Treatment started on December 8, 1951 and continued for 42 days, 113 gm. There was an immediate symptomatic response with rapid clearing of roentgenogram. Her sputum is now negative for tubercle bacilli on concentrate. She continues at rest.

A. Roentgenogram taken December 4, 1951.

B. Roentgenogram taken January 17, 1952.

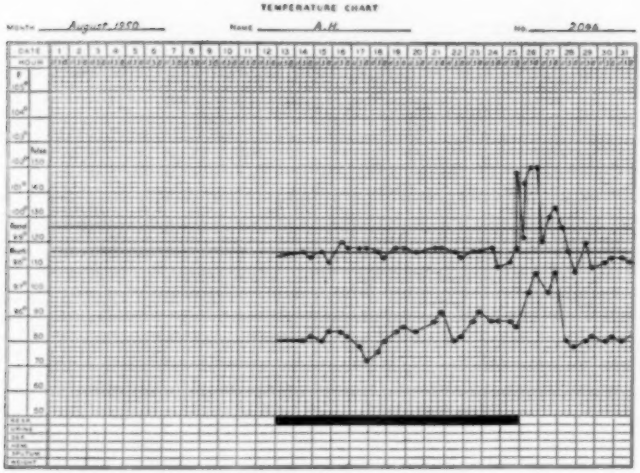
Temperature chart indicating commencement of pyrazinamide therapy.



Case 9 (J. F.)

FIG. 11. Thirty-four-year-old male with onset of tuberculosis in 1936. Tuberculosis inactive many years. Increasing nonprotein nitrogen, blood pressure, and fever, and subcutaneous nodules for several months, with diagnosis of periarteritis nodosa proved by biopsy. Treatment started on January 19, 1950 and continued for six days. No symptomatic response to pyrazinamide therapy. Death from periarteritis several months later.

Temperature chart indicating commencement of pyrazinamide therapy.



Case 27 (A. H.)

FIG. 12. Thirty-seven-year-old female. A diagnosis of tuberculosis was made in October 1949. The patient was admitted in Feburary 1950. Her roentgenograms showed scattered infiltration above the third rib bilaterally. She was asymptomatic. Her sputum was intermittently positive for tubercle bacilli. Roentgenograms showed little or no change on five months' bed rest. Pyrazinamide was started on August 14, 1950. No change was seen until a sudden chill sent her fever to 102°F. on the eleventh day. (Subsequently more intense tanning of the skin was noted). The treatment was stopped but started two weeks later at her request without incident. Total amount of drug, 65 gm., in 37 days. Her subsequent roentgenograms showed some clearing bilaterally. Her sputum was negative for tubercle bacilli on concentrate and five cultures. She was discharged and remains inactive.

Temperature chart indicating commencement of pyrazinamide therapy.

STREPTOMYCIN IN MINIMAL TUBERCULOSIS[1]

ETHEL FLOREY

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INTRODUCTION

Since the publication of the results of the first clinical trials of streptomycin in pulmonary tuberculosis in 1946 by Hinshaw, Feldman, and Pfuetze (2), it has been plain that the most dramatic results are obtained in relatively acute exudative disease. The use of streptomycin in minimal tuberculosis was deprecated because efficient forms of treatment were already available; and the fact, which emerged later, that resistant strains might appear during treatment strengthened the objection. If such strains appeared early in the course of therapy, the patients might derive no benefit from the treatment, while at the same time they would have prejudiced their chances for obtaining benefits from streptomycin treatment in the future when they might need it more urgently (11). Moreover, the ever-present possibility of infecting others with streptomycin-resistant tubercle bacilli is particularly likely with patients with minimal lesions, for they are so much more apt to be ambulatory than patients with more advanced disease.

Nevertheless, with penicillin in mind, in 1947 it was considered useful to see if the administration of streptomycin to early cases of tuberculosis would so lessen or prevent damage as to reduce expenditure of the drug, hospital accommodation, invalidity, and medical attendant's time.

At that time, before the effect of administration of para-aminosalicylic acid (PAS) with streptomycin on the emergence of resistant strains of tubercle bacilli was known, the problems were to formulate how patients should be treated without running the risk that the strains of *Mycobacterium tuberculosis* they harbored would be replaced by drug-resistant strains while full control of the infection was being obtained, and to devise a criterion of effectiveness of treatment. Progress, never easy to assess roentgenographically, was especially difficult when the size of the original lesion might be little bigger than the area of scarring left after healing had taken place. Bacteriologic control was also thought to be of questionable value because patients in whom the diagnosis of minimal tuberculosis is made frequently have no sputum, and cultures positive for tubercle bacilli are often obtained only intermittently, if at all. It was therefore considered essential that "controls" should be observed for the same period as patients treated with streptomycin. The course of tuberculosis in these patients treated by the methods considered most suitable before the introduction of streptomycin should form the necessary standard against which progress in streptomycin-treated cases might be assessed.

A visit to the United States in 1947 provided an opportunity of learning at

[1] From the Central Middlesex Hospital, London, and Sir William Dunn School of Pathology, University of Oxford.

first hand the experience of workers who had already been using streptomycin for two years and also of approaching pharmaceutical firms,[2] who generously supplied the streptomycin for such an investigation when it was not generally available in Great Britain.

Accordingly, an investigation was started with the inclusion of "control cases" which received no streptomycin. For various reasons which are mentioned below, it was not possible to complete the study. As a consequence, the following account represents, in effect, a report of the clinical, bacteriologic, and roentgenographic course of minimal tuberculous lesions of the lung during and subsequent to streptomycin compared with older therapy over the first two years of observation.

MATERIALS AND METHODS

Original Scheme of the Investigation

Definition of minimal tuberculosis: The definition of minimal tuberculosis was similar to that adopted by the American National Tuberculosis Association (1)[3] and the Prophit Tuberculosis Survey (13). A lesion was considered to be minimal when, as represented on anteroposterior roentgenograms, its total extent (one or more areas) was no greater than that extending from the apex of the lung to the lower border of the second rib, and was present in association with positive bacteriologic evidence of tuberculous infection. It was not found practicable to exclude demonstrable excavation, as tomographs so frequently revealed small, soft-walled cavities. The duration of symptoms before treatment was not longer than a year.

Patients were to be obtained in the following manner: First, they would be diagnosed roentgenographically at the Willesden Chest Clinic and then sent for admission as in-patients by the senior tuberculosis officer to the tuberculosis unit of the Central Middlesex Hospital, where bacteriologic confirmation of the diagnosis was to be made.

Selection for "control" or streptomycin series: Selection for "control" or streptomycin series would be on the basis of alternate cases according to the date when the first positive bacteriologic specimen had been collected. This rule was strictly kept, with the result that acceptance for one or the other series did not necessarily follow the order of admission to the hospital and was therefore not known to the physician. An immediate result from examination of a smear of sputum might be obtained, or six weeks might elapse before the result of the culture was known which admitted or excluded the case from the investigation. During this time a third patient might have been admitted whose inclusion in one or the other series would also depend on when the results of examinations of previous patients were obtained.

General treatment: "Control cases" were to be treated, when possible, according to recognized lines of collapse therapy, but patients were to be included among them even when collapse was impracticable. Streptomycin treatment is discussed later, under "the scheme in operation," in which both types of treatment are dealt with in greater detail.

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[2] Merck & Co., Inc., Charles Pfizer & Co., Inc., and E. R. Squibb & Sons have kindly provided the supplies of streptomycin for this project through the instrumentality of Mrs. Mary Lasker of New York City.

[3] Definition: Slight lesions without demonstrable excavation confined to a small part of one or both lungs. The total extent of the lesions, regardless of distribution, shall not exceed the equivalent of the volume of lung tissue which lies above the second chondrosternal junction and the spine of the fourth or body of the fifth thoracic vertebra on one side.

Investigations: Serial investigations to be carried out were as follows:

Symptoms
Roentgenograms of chest
Bacteriologic examinations
Weight measurements
Erythrocyte sedimentation rate
Hemoglobin concentration
Lymphocyte-monocyte ratio

To reduce bias, all observations except estimation of the patients' symptoms were to be made by persons other than the present investigator, the latter merely recording them. This seemed particularly important when the roentgenograms were concerned. The team of radiologists attached to the hospital were to report on the films throughout, even when the patients had ceased to be in-patients and were attending the Willesden Chest Clinic.

In each series, cases were to be followed for a minimum period of five years from the time of each patient's admission to the hospital.

The Scheme in Operation

Number of cases: Forty-eight patients considered to have minimal pulmonary tuberculosis were admitted during the two-year period which started in March 1948. This should have meant 24 patients in each series, but 12 were lost before admission to either series for the following reasons:

One patient was excluded because the diagnosis of a minimal lesion was not confirmed by the radiologists and the history was of chronic tuberculosis treated for four years.

Five patients had to be removed from the series because cultures and guinea pig inoculations remained persistently negative.

Four patients, who did not have complete bacteriologic examinations before treatment was started, were subsequently persistently "negative."

Two patients were withdrawn by attending physicians, once streptomycin was freely available, and given both chemotherapy and collapse.

Of the remaining 37 patients, 18 served as "controls" and 19 were treated with streptomycin. Unfortunately, however, 10 of the total series were also lost after treatment and follow-up had proceeded for three months to two years.

Among the streptomycin-treated cases: one patient early in the series, whose sputum had not remained consistently negative for *M. tuberculosis* after two courses of streptomycin, elected to be treated by collapse; 3 patients were removed by their physician after six, fifteen, and twenty-four months, respectively, and treated by collapse; and one patient left the district after a year and could not be traced.

Among the "control" cases: 2 patients left the district; one, after three months, for a mental hospital where conditions might not have been comparable with the conditions of other patients. The second went to Scotland, whence no report was obtained from his tuberculosis officer; and 3 patients, who after two years were not progressing to the satisfaction of their tuberculosis officers, were given streptomycin and PAS.

OBSERVATIONS

Status of Tuberculosis at the Beginning of Treatment

Duration of symptoms: Two patients in each series had been contacts of tuberculous friends or members of a tuberculous family in past years. They had been examined roentgenographically at regular intervals and, although each had had a small shadow for several years, this had remained stationary. These patients had no complaints, and the duration of their symptoms was calculated from the

TABLE 1

DURATION OF SYMPTOMS BEFORE TREATMENT

Number of Cases

SERIES	TOTAL	UP TO 2 MONTHS	3 TO 5 MONTHS	6 TO 12 MONTHS
"Control"	18	6	5	7
Streptomycin-treated	19	5	9	5

TABLE 2

AGES AT THE BEGINNING OF TREATMENT

Number of Cases

SERIES	TOTAL	15 TO 20 YEARS	21 TO 35 YEARS	36 TO 49 YEARS
"Control"	18	7	6	5
Streptomycin-treated	19	4	11	4

TABLE 3

BACTERIOLOGICAL EXAMINATION BEFORE TREATMENT

Number of Cases

SERIES	TOTAL	SMEARS AND CULTURES POSITIVE FOR TUBERCLE BACILLI	CULTURES ONLY POSITIVE FOR TUBERCLE BACILLI	SMEARS POSITIVE FOR ACID-FAST BACILLI*
"Control"	18	5	10	3
Streptomycin-treated	19	10	8	1

* Cultures negative or not done. No positive specimens after admission.

first time an increase in the size of the shadow was observed. The remainder had some symptoms, such as a "feverish cold," malaise, attack of indigestion, or expectoration of blood-stained sputum, from which they dated their complaint. With these not very clear-cut criteria, it was possible to give a period for the duration of symptoms which, though approximate, gave some idea of the acuteness of the activity in each series. The cases in the streptomycin series were, on the whole, more acute than those in the control series. These data are presented in table 1.

Age and sex distribution: The sex distribution was approximately equal: there

were 7 males among the 18 "controls" and 8 among the 19 streptomycin-treated cases. The age distribution in the two series was not grossly dissimilar, as is shown in table 2.

Severity of infection: From the above material and the bacteriologic and radiologic data shown in tables 3 and 6, it was possible to estimate the relative severity of the disease in the two series. In the streptomycin series there were 2 less patients with purely exudative lesions than in the "control" series; 2 more with infraclavicular lesions (found to have the worst prognosis in the Prophit Survey in Great Britain (13)); 2 more with demonstrable cavities; 5 more who were coughing up sputum in which tubercle bacilli were readily demonstrated; and 7 more in whom there was evidence of anemia. There was also one patient who had an anal fistula. The factors weighing against the "controls" were the presence of one more patient with bilateral disease (lost to the series within twelve months) and one more than in the streptomycin-treated cases with two foci on one side; one patient had fluid in the pleural cavity. In 3 more patients the lymphocyte-monocyte ratio was lower than the arbitrary standard of normality, 90 to 10. The initial condition was not considered therefore to favor the streptomycin series.

Treatment

Control cases: Pneumothorax was induced in 14 patients, pneumoperitoneum in 7. In 2 patients with fever, pneumoperitoneum preceded pneumothorax; and in 2 patients, the procedures were performed concurrently. In one patient collapse was impracticable owing to an effusion.

Collapse was induced within the first two weeks in the majority of the patients, but it was delayed in 3 due to fever or to cavitation which appeared to be enhanced when collapse was first attempted. Refills were performed first at weekly, later at fortnightly, and eventually at three-weekly, intervals, collapse being maintained for ten months in 2 patients and for two years or more in 15 patients.

These patients, except for one with a lower lobe which appeared completely collapsed before discharge, remained in the Unit between three and six months. They were then transferred to a sanatorium, convalescent home, or their own homes, the type of home rather than the patient's condition dictating the place of convalescence. Here observation and treatment were continued until patients could attend the Willesden Chest Clinic for the continuance of collapse therapy. The initial period of institutional treatment averaged 19.8 weeks per patient (9 to 44 weeks).

Streptomycin-treated cases: Patients did not necessarily receive chemotherapy within the first week after admission to the hospital, though there was no therapeutic reason for deferring it. However, the difficulties of organization did not prevent first administration of the drug to all patients by the end of the third week.

The original scheme of treatment was based on two considerations: the need to eliminate all evidence of infection before treatment was abandoned and,

equally essential, the need to avoid inducing the emergence of resistant strains of *M. tuberculosis*. With these points in mind, treatment was divided into separate courses, each of 0.5 gm. of streptomycin twice daily and limited to twenty-eight days. At the time the investigation was begun and during the following year, no report was published of the appearance of resistant strains earlier than twenty-nine days from the start of streptomycin, although they had been evident between the twenty-ninth and thirty-fourth days (5, 6, 11, 15, 16, 18).

Because of the number of patients with acid-fast bacilli in smears of their sputa, it soon became clear that a single course of twenty-eight days was not sufficient to eliminate the organisms, and subsequent analysis showed that in only 5 of the 19 patients were cultures of swabs or sputum consistently free after the first course. It was therefore decided that at least two courses were essential for each patient, but the second was not given until a bacteriologic report was received on a specimen obtained after treatment had ceased. The interval between the courses was never less than six weeks and was frequently longer because of the appearance on the culture media of a colony of tubercle bacilli requiring a test of sensitivity. If the culture was negative or (if positive) the bacilli were found to be sensitive to streptomycin, the second course of twenty-eight days was begun. After this, the administration of further treatment was guided by the bacteriologic examinations and roentgenographic evidence of renewed activity and, in women, by the onset of pregnancy and parturition. When indicated by these signs, injections of streptomycin were given at home by the district nurse, a single daily injection of 1 gm. being used and two courses advised. In this way streptomycin-treated patients remained under observation and might receive courses of treatment for as long as collapse was maintained in "control" patients.

All institutional treatment was given in the Unit so that a more rigid bacteriologic check could be kept on the patients. The initial period of institutional treatment averaged 22.8 weeks per patient (9 to 43 weeks). Each patient, therefore, had, on an average, the advantage of three weeks longer under skilled supervision than did the "controls."

After discharge from the hospital, few patients received the treatment recommended, for many personal factors entered into the periodic assessment of each case. The fear that a new type of treatment might be prejudicial to the patient's welfare when a well-tried one was available, and reluctance to rely for indications of activity on bacteriologic reports unsupported by roentgenographic signs, influenced tuberculosis officers to withhold streptomycin treatment when it was advised or to substitute collapse therapy more or less empirically.

Such difficulties led to the abandonment of the systematic investigations outlined above and of the administration of streptomycin alone on December 12, 1950, when the patients had been under observation for six months to two years. The systematic investigations outlined above ceased abruptly, and patients were thereafter treated on the recommendations of the tuberculosis officers only. It was, however, eventually possible for the present investigator to obtain reports of patients' clinical progress and bacteriologic examinations. Treatment

in some cases was continued along lines similar to those already laid down with the addition of para-aminosalicylate to the chemotherapy, but the intervals between courses of streptomycin were much greater and the drug was seldom given again until there was roentgenographic evidence of spread.

Because of the difficulties in continuing the investigation, it has been thought advisable to report the progress of cases over the first two years, although it was obvious that full control of the infection could not necessarily be assured by then.

Course During and After Therapy

Bacteriologic: Approximately one-half of the patients had no recognizable cough or sputum on admission and it was only with some struggle that they were able to cough up a sample for examination. It was found better to obtain laryngeal swabs from these patients, as otherwise they were apt to spit saliva into their specimen jars. Gastric washings were frowned on by staff and patients alike. Four "control" and 8 streptomycin-treated patients had no other type of specimen collected throughout the period of observation, but there was a higher proportion of streptomycin-treated patients from whom sputum containing demonstrable acid-fast bacilli in direct smears was obtained than there was with the controls (table 3).

Methods

Laryngeal swabs: Cotton-wool swabs, dipped in sterile distilled water before collection of the sample, were then rubbed in 5 ml. of 10 per cent hydrochloric acid in a centrifuge tube. After standing at room temperature for fifteen minutes, the acid was neutralized with 4 per cent sodium hydroxide containing phenol red and centrifuged for fifteen minutes. The supernatant was poured off, and the deposit was inoculated on Löwenstein egg medium and incubated for six weeks at 37°C. If the cultures were negative, they were not discarded for eight weeks.

Sputum: To 5 ml. of sputum in a wide-mouth 30 cc. universal container, 5 ml. of 10 per cent hydrochloric acid were added. The container was then shaken at room temperature for fifteen minutes in an electric shaking machine. After neutralizing with 4 per cent sodium hydroxide, the contents were centrifuged for fifteen minutes at 3,000 r.p.m. Smears and cultures on Löwenstein egg medium were made from the deposit.

Sensitivity tests: Ten ml. of Dubos-Davis Tween[4]-albumin medium were inoculated with growth from Löwenstein culture; 0.2 ml. amounts of the growth resulting after incubation for twenty-one days were used to inoculate tubes, each containing 10 ml. of the same medium with different concentrations of streptomycin incorporated in them. The concentrations of streptomycin were 0.25, 0.5, 1.0, 10, 1,000, and 5,000 γ per ml. A control tube without streptomycin and also a parallel series of tubes with the strain H37Rv were inoculated. After incubation for twenty-one days, the lowest concentration in which no growth appeared was recorded as the sensitivity of the organisms (recommendations as in (6)).

In figures 1 and 2 are presented the monthly bacteriologic findings in each series, together with the periods when active treatment was applied. For the

[4] Proprietary name of the polyoxyethylene derivative of Sorbitan monooleate, Atlas Powder Co., Wilmington, Delaware.

sake of clarity, pneumothorax refills are not shown in figure 1, the boxed-in areas signifying the period of time during which collapse was maintained. During the first six months, when patients were usually in the hospital, the majority of

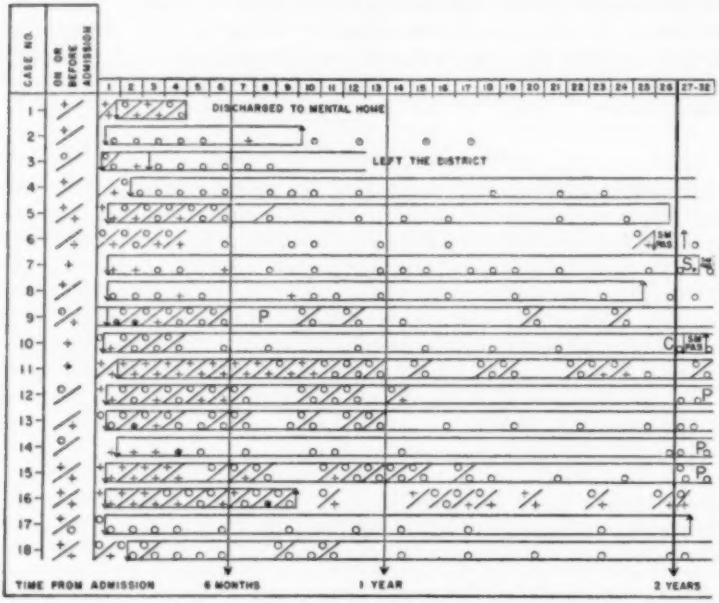
CASE NO.
ON OR BEFORE ADMISSION
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27-32
DISCHARGED TO MENTAL HOME
LEFT THE DISTRICT
TIME FROM ADMISSION
6 MONTHS
1 YEAR
2 YEARS

FIG. 1. *"Control cases."* Results of bacteriologic examinations at months from first admission.

Plus signs indicate presence of acid-fast bacilli on smear or *M. tuberculosis* on culture. The diagonal across a square separates the report of the smear (above), if sputum was present, from that of the culture (below).

↓ = start of collapse or streptomycin
↑ = discontinuance of collapse or streptomycin
* = incubator overheated; culture result not known
SM = streptomycin
PAS = para-aminosalicylate
P = parturition
H = hemoptysis
C = cavity on roentgenogram; no bacteriologic result known
Sp = spread on roentgenogram; no bacteriologic result known
R = resistant to streptomycin
A-P = artificial pneumothorax induced in a streptomycin case
L = lobectomy
Results as available on December 31, 1951.

specimens were taken weekly. If any were "positive," the sign indicating the monthly result was made "positive," irrespective of the number of other "negative" results.

Four hundred and forty-seven examinations were made of the "control cases" and 650 of the cases in the streptomycin series. The opportunities for detecting

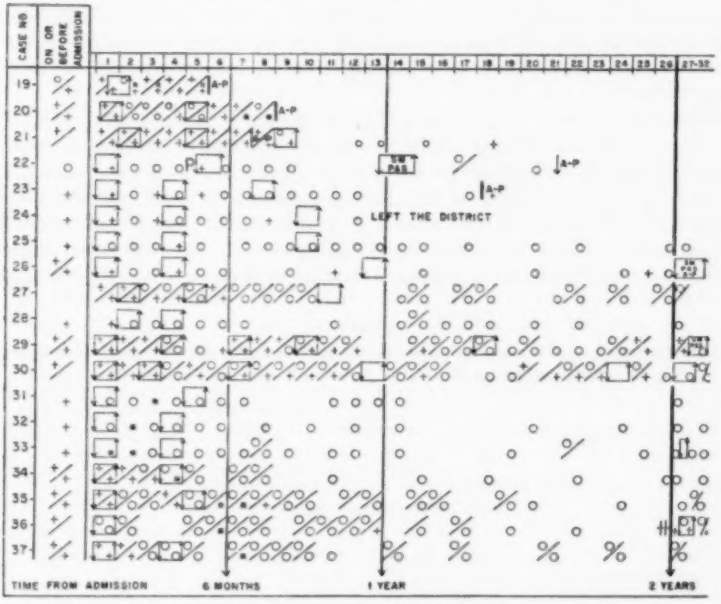

FIG. 2. *Streptomycin-treated cases.* Results of bacteriologic examinations at months from first admission.

Plus signs indicate presence of acid-fast bacilli on smear or *M. tuberculosis* on culture. The diagonal across a square separates the report of the smear (above), if sputum was present, from that of the culture (below).

For convenience of charting, the streptomycin treatment is placed within the month to which it most closely corresponds.

↓ = start of collapse or streptomycin
↑ = discontinuance of collapse or streptomycin
* = incubator overheated; culture result not known
SM = streptomycin
PAS = para-aminosalicylate
P = parturition
H = hemoptysis
C = cavity on roentgenogram; no bacteriologic result known
Sp = spread on roentgenogram; no bacteriologic result known
R = resistant to streptomycin
A-P = artificial pneumothorax induced in a streptomycin case
L = lobectomy

Results as available on December 31, 1951

a positive result were therefore nearly one and one-half times as great in the latter as in the "controls." In table 4 it may be seen that by the end of the first three months, during which time examinations were relatively evenly

divided, the number of cases in which cultures for *M. tuberculosis* had become negative was no greater among the streptomycin series than among the controls. Subsequently the preponderance of cases showing positive cultures for *M. tuberculosis* among the streptomycin series had less significance because of the greater number of samples examined. By the end of two years, cultures positive for *M. tuberculosis* were obtained from an equal proportion of cases in each series.

Immediate effect of streptomycin: The immediate effect of streptomycin administration was much more obvious when weekly instead of monthly reports were studied. At the end of the first course, sputum smears showed diminution or absence of organisms in every case, and cultures had become negative for tubercle bacilli in half of the cases. Reversal of infectiousness continued after administration of the drug had ceased until no more than 2 of the 18 cultures tested ten weeks after the beginning of therapy were positive for *M. tuberculosis*.

TABLE 4

COMPARISON OF THE NUMBERS OF CASES IN WHICH ONE OR MORE CULTURES POSITIVE FOR TUBERCLE BACILLI WERE FOUND AT VARIOUS TIMES FROM FIRST TREATMENT

SERIES	AT END OF THIRD MONTH		BETWEEN THIRD AND SIXTH MONTHS		BETWEEN SIXTH MONTH AND ONE YEAR		BETWEEN ONE AND TWO YEARS	
	Total	Positive	Total	Positive	Total	Positive	Total	Positive
Number of cases								
"Control"	18	4	18	8	17	5	15	4
Streptomycin-treated	19	5	19	10	18	7	15	4
Number of cultures								
"Control"	210	82	79	21	68	16	59	10
Streptomycin-treated	207	94	169	38	125	29	90	11

Comparison with "controls" showed that a corresponding reduction in positive cultures had taken place, only 4 of 16 remaining positive for tubercle bacilli at this time. From the tenth week, if no further streptomycin was given, the number of cultures positive for *M. tuberculosis* slowly increased. This was an additional reason for giving the second course in the tenth week. Moreover, appearance of these positive cultures made it hard to withhold therapy until the result of the drug-sensitivity test was known, as concurrent deterioration in the patient's condition was sometimes observed. Nevertheless, at this stage the rule was rigidly observed and eventually had its reward in the knowledge thereby obtained.

The most striking difference between figures 1 and 2 is the relatively small amount of active treatment required by the streptomycin-treated patients to achieve results apparently equivalent to those obtained with two or more years of continuous collapse therapy. One of the most significant findings of the whole study was the infrequency with which drug-resistant strains of tubercle bacilli

were isolated from the streptomycin-treated cases. All positive cultures were tested for drug sensitivity, and comparable studies from the "control cases" formed a background where the degree of variation might be judged. In these studies of the "control cases," sensitivity tests of 128 cultures from different samples were made. The minimal inhibiting concentration varied between 0.25 and 0.8 γ per ml. or 0.4 to 1.8 times the drug resistance of the strain H37Rv which was tested simultaneously. In individual patients the widest range was similar: 0.3 to 0.8 γ per ml.; but usually it remained within a twofold variation.

Among the streptomycin series, one patient produced strains resistant to 5,000 γ per ml. within the second half of the four weeks during which he received his first course of treatment and at a time when a cavity with fluid level had become apparent. Two others discharged tubercle bacilli which showed evidence of decreasing sensitivity. In one patient, the sensitivity of the strain isolated decreased from 0.5 to 2.0 γ per ml. (or four times the resistance of H37Rv) during her second course, and decreased twofold during the interval following it to 5.0 γ per ml. (ten times the resistance of H37Rv). This occurred at a time when a cavity became manifest, but no further decrease in the drug sensitivity of her cultures occurred during her final course of streptomycin, during which her sputum became negative for *M. tuberculosis* and remained so for the following six months. Later, another "positive" culture, which appeared while she was undergoing collapse therapy, was still inhibited by 5 γ of streptomycin per ml. From the gastric washings of the other patient, obtained ten months after her third course of streptomycin, a culture was isolated which was sensitive to only 10 γ per ml.

In the remaining 14 patients from whom *M. tuberculosis* could be cultured, no strains which required more than 1.0 γ per ml. for inhibition were found among 151 separate cultures. These findings are summarized in table 5.

Some of the positive cultures appeared only many months after the course designated and the time the sensitivity tests were done had no special relationship to the time after the treatment was completed, but depended on the occasional appearance of colonies on the culture medium; there were seldom many, except in the first six months of treatment; and they often appeared late on the culture tube.

Four patients in the series did not receive another course of streptomycin despite the fact that the bacteriologic findings indicated that they still harbored drug-sensitive tubercle bacilli. In 3 of these 4 patients, more obvious signs of activity developed later and provided the necessary stimulus for further streptomycin administration.

One patient (Case 36, figure 2[4]), who had only one course of streptomycin, had 11 successive negative cultures of his sputum, including a "negative" guinea pig inoculation test, during the following ten months. Nevertheless, near the close of the first year of observation and at a time when the roentgenographic report indicated that calcification was progressing and the result satisfactory, a single specimen gave culture positive for *M. tuberculosis* and a guinea pig inoculation test was also "positive." As no other sign of reactivation was found, streptomycin was withheld. During the subsequent year, he married and

[4] Patients are described by case numbers in order to facilitate reference to the charts.

started to work, but remained thin and his complexion was poor. At the end of the second year he had an hemoptysis and subsequently, when bacteriologic examinations were made, cultures positive for tubercle bacilli were obtained. This patient has since had two courses of streptomycin but, unfortunately, they were fifteen weeks apart. Nevertheless, at the time he was last observed, four years after he was first seen, he had had three successive cultures negative for tubercle bacilli, was in good health, and was working full time.

TABLE 5

MINIMAL INHIBITING CONCENTRATION OF STREPTOMYCIN FOR STRAINS OF MYCOBACTERIUM TUBERCULOSIS ISOLATED AFTER SEPARATE 28-DAY COURSES OF STREPTOMYCIN ALONE TO 19 PATIENTS

(Micrograms per Milliliter Required to Inhibit Growth Completely in Dubos-Davis Medium)

CASE NUMBER	ON ADMISSION	AFTER FIRST COURSE	SECOND	THIRD	FOURTH	FIFTH	SIXTH
19	0.5	5,000	*				
20	0.2	0.3	0.5	*			
21	0.25	0.5	5.0	5.0	*		
22	0.5	0.5	N.D.	†			
23	0.5	0.5	N.D.	10.0	*		
24	0.25	0.5	0.5	—			
25	0.5	—	0.5	—			
26	0.25	—	0.5	UK	†		
27	0.25	0.5	0.5	—			
28	0.5	—	—				
29	0.2	0.3	0.5	1.0	0.5	1.0	†
30	0.5	0.5	0.5	0.5	0.5	0.5	
31	0.2	—	—				
32	0.3	—	0.5	1.0			
33	0.4	—	—	—			
34	0.3	0.3	—				
35	0.3	0.3	0.3				
36	UK	0.5	0.5	0.5	—		
37	0.3	0.3	—				
Number found positive for *M. tuberculosis*		13	13	7	2	2	0

* = changed to collapse therapy with or without streptomycin and PAS.

† = changed to streptomycin and PAS (after one year in case 22; after two years in cases 26 and 29).

N.D. = not done.

— = no positive culture obtained after last course.

UK = unknown.

Blank spaces indicate no further courses of streptomycin given.

In Cases 30 and 29, isolated cultures positive for tubercle bacilli appeared eight and twenty-four weeks, respectively, after their last course of streptomycin.

In Case 30, repeatedly "positive" sputum cultures, with acid-fast bacilli also appearing in smears, were not demonstrated until nineteen weeks later. The patient had two further courses of streptomycin during four months, and remained free of "positive" cultures for the following twelve months, the last cultures being negative for tubercle bacilli on guinea pig inoculation.

The other patient (Case 29) appeared to be well on the way to recovery after an ischio-rectal abscess, from whose walls sections typical of tuberculous granulation tissue were cut and tubercle bacilli cultivated. She was active in her house, symptomless, and devoid of sputum, and cultures of laryngeal swabs were "negative" for six months when a specimen of sputum was obtained in which tubercle bacilli were demonstrated on film and in culture.

TABLE 6

ROENTGENOGRAPHIC CHANGES

ROENTGENOGRAPHIC CONDITION BEFORE TREATMENT	NUMBER OF CASES	
	"Control"	Streptomycin-treated
Site		
Apical lesions	8	8
Upper lobe lesions, below clavicle	6	8
Mid-zone lesions	—	2
Apex and mid-zone	2	1
Apex and base	1	—
Basal lesions	1	—
Total	18	19
Number of lesions		
Single focus	13	16
Two foci, one side	2	1
Two foci, bilateral	3	2
Total	18	19
Type of lesion		
Exudative	10	8
Exudative superimposed on fibrosis or scarring	1	2
Consolidation	1	3
Infiltration	6	4*
Various, described as "increased density" or "mottling"	—	2
Total	18	19
With cavitation	7†	9†
With fluid	1	—

* One with scarring.

† Includes those described as small areas of breakdown, recognized as cavities in tomographs.

Unfortunately, this patient's sputum was not examined again for seventeen weeks, nor was she given any streptomycin. At the time of this examination, her sputum was found to be repeatedly positive for tubercle bacilli. A sixth course of streptomycin, to which was added sodium para-aminosalicylate, resulted in cultures negative for tubercle bacilli during the following eight months.

Another patient (Case 35) appears to have been able to deal with his tubercle bacilli for, although thin, he has remained well, active, and at work for two and one-half years since two colonies of tubercle bacilli were detected at one examination. Cultures negative for

tubercle bacilli have since been repeatedly obtained from laryngeal swabs, though there has been little clearing of the apical shadows.

Roentgenographic Course During and After Therapy

The roentgenographic diagnosis of tuberculosis was initially made on the appearance of the lungs in anteroposterior films. Taken as a whole, there was no gross difference between the two series. In table 6 may be seen the description of the findings as made in the radiologists' reports.

Much attention was paid to the examination of serial roentgenograms, which are usually considered to provide the most important indication of progress. Unfortunately, the type of treatment given to the "control cases" precluded a

TABLE 7

COMPARISON OF ROENTGENOGRAPHIC APPEARANCES AT DIFFERENT INTERVALS WITH APPEARANCES AT THE BEGINNING OF TREATMENT

Number of Cases

TIME FROM BEGINNING OF TREATMENT	TOTAL EXAMINED	IMPROVED	UNCHANGED	DETERIORATED	NOT COMPARABLE
At end of 3 months					
"Control"	18	1	11	3	3
Streptomycin-treated	19	4	13	2	
3 to 6 months					
"Control"	17	3	10	3	1
Streptomycin-treated	19	2	11	5	1
6 months to one year					
"Control"	16	3	10	3	
Streptomycin-treated	16	8	6	2	
2 years					
"Control"	15	6*	4	4	1
Streptomycin-treated	13†	9	1	3	

* 2 not strictly comparable.

† Of the 6 streptomycin-treated cases excluded, 5 had had their treatment changed and one had left the district.

fair running comparison, for changes were much more readily detected in the expanded lung of the streptomycin-treated cases than in the collapsed lung of "controls." Frequently the radiologist, in spite of every effort, was unable to decide whether or not any change had taken place in the "controls." An approximation is given in table 7 based solely on the radiologists' reports, as they seldom saw the patients and were unlikely to be influenced by personal factors.

The dramatic changes seen by other workers in acute exudative or miliary tuberculosis were seldom seen in these minimal lesions and, when observed, could be paralleled by similar though less rapid changes in "control cases." Gradual changes were more common and often little difference was noted in the roentgenographic appearance of apical lesions on admission and one to two years after treatment first began, in spite of the patients' steady progress back

to health and full-time work during this period. No roentgenographic change was noted in 4 cases, previously described, for some months after a culture positive for *M. tuberculosis* had been obtained from a laryngeal swab. One of these patients, in the absence of further streptomycin treatment, after a "positive" culture in the thirteenth month subsequently had an hemoptysis, signs of extension of the lesion, and the persistent discharge of bacilli in the sputum until two more courses of streptomycin were given (Case 36).

The fluctuations between deterioration and improvement from period to period among the streptomycin series are evidence of the difficulty of interpreting progress in these minimal lesions but may also indicate that an early spread of disease may be quickly controlled by streptomycin. One patient, in whom spread of disease was reported from observation of a film taken at Willesden Chest Clinic, was put to bed and given a course of streptomycin. When this

TABLE 8

BLOOD EXAMINATIONS BEFORE TREATMENT

Number of Cases

SERIES	TOTAL	ERYTHROCYTE SEDIMENTATION RATE*		HEMOGLOBIN PER CENT		LYMPHOCYTE-MONOCYTE RATIO†	
		>10	<10	100 or more	<100	90:10 or more	<90:10
"Control"	18	10	8	9	9	6	10
Streptomycin-treated	19	11	8	3	16	10	7

* Westergren, read at one hour.

† 2 in each series unknown.

patient was re-examined one month later on her reporting to the Central Middlesex Unit, the radiologist reported no change in the shadow compared with that in the roentgenogram taken five months previously.

It is unfortunate that the premature end of the investigation has prevented comparison with the "control" series after expansion of the patients' lungs. Comparison of the roentgenographic appearances on admission and after one year, however, showed that 9 of 16 streptomycin-treated patients and 3 of the 16 "controls" had shown improvement in their lesions, and 3 streptomycin-treated and 4 "control" patients had shown progression. Nevertheless, these results in the streptomycin series did not warrant any relaxation of vigilance in the observation of patients for any indication of renewed activity and for consequent treatment.

At the end of two years, though fewer cases were available for study, the proportion showing improvement had risen. A few more years, however, would have been necessary to make certain that the improvement was permanent. Throughout the two years, cavities other than those seen on admission had become visible in 6 "control" and in 4 streptomycin-treated cases.

Hematologic Studies

In table 8 the results of blood examinations of patients on admission to the hospital are shown. The only marked difference appeared in the concentration of hemoglobin, the average being 98 per cent of the normal value in the "controls" and 88 per cent in the streptomycin-treated patients.

Erythrocyte sedimentation rate: In both series there was only slight acceleration in the erythrocyte sedimentation rate. An average increased acceleration of 10 mm. per hour during the first course of streptomycin was noted and was greatest in patients whose lesions were predominantly exudative. After collapse was introduced, on the other hand, there was less change and, in the reverse direction, an average retardation of 2 mm. was observed. During subsequent courses of streptomycin, acceleration of fall was less noticeable. After a year

TABLE 9

COMPARISON OF BODY WEIGHT AFTER VARIOUS INTERVALS WITH WEIGHT ON ADMISSION

SERIES	TOTAL NUMBER OF PATIENTS	NUMBER IMPROVED	NUMBER LOST	NUMBER NOT KNOWN	AVERAGE GAIN OVER WEIGHT ON ADMISSION (IN POUNDS)
At the end of three months					
"Control"	18	13	5		4.17
Streptomycin-treated	19	16	2	1	9.5
Between three and six months					
"Control"	17	13	4		11.0
Streptomycin-treated	18	14	4		14.5
Between six months and one year					
"Control"	15	11	4		10.0
Streptomycin-treated	17	11	4	2	13.5
Between one and two years					
"Control"	16	12	4		11.5
Streptomycin-treated	15	12	3		14.2

there was no significant difference between the series; 8 "control" and 10 streptomycin-treated patients still had erythrocyte sedimentation rates of more than 10 mm. per hour, but after two years a higher proportion of streptomycin-treated patients had normal rates. As tests were carried out on only 9 patients of each series, however, not much reliance can be placed on these results.

Lymphocyte-monocyte ratio: The lymphocyte-monocyte ratio also showed a tendency to change during streptomycin treatment, the ratio falling during administration and rising again approximately two weeks after therapy had been discontinued. During the first year, however, in both series, the ratio had risen to more than 90 to 10 in an equal number (10 cases); and at the end of two years all of 15 streptomycin-treated patients tested had reached this standard, as had all of those tested among the 10 "control" patients. Unfortunately, 2 "control" patients who had had roentgenographic relapses by this time had not been tested during the previous six months or more.

Hemoglobin concentration: In spite of the fact that 16 streptomycin-treated patients had hemoglobin concentrations less than 100 per cent of the normal value, in only 3 did the concentration remain below this level at the end of three months, and in only one at the end of six months. In none of the 9 patients tested after two years was the concentration of hemoglobin less than 100 per cent of normal. In the "controls," whose average hemoglobin concentrations before treatment were 98 per cent of normal as against 88 per cent in the streptomycin series, there was less room for improvement. When present, it was slower, for 5 of the 9 originally with subnormal values remained so at the end of three months and throughout the following two years.

Changes in Body Weight

Changes in body weight were also more marked in the streptomycin series. The number of patients who gained and maintained an increase over their weight on admission to the hospital are shown in table 9.

General Considerations

It may be useful to discuss the treatment and progress of the streptomycin-treated cases further. Duration of treatment was not limited to any specific period but was adjusted to individual requirements, two courses of treatment being recommended whenever any sign of activity was found, provided the organism, if found, was sensitive to streptomycin *in vitro*.

One patient (Case 27), with one of the more extensive lesions of the series, as shown on the anteroposterior film, received no more than three courses of treatment, completed within ten months. He had remained well and at work, with a gradually clearing roentgenographic film and sputum negative for tubercle bacilli, from the end of the last course of treatment throughout the remainder of the two years since admission.

In another patient, bilateral apical lesions have, for three years, shown no roentgenographic sign of clearing or extending (Case 35); yet he has had two courses of streptomycin only, completed within five months, and has been at work, feeling well, without fatigue, and with cultures persistently negative for tubercle bacilli for more than two years.

Cases 29 and 30 (table 5) are particularly instructive because both patients have had recurrent "positive" cultures of their sputum between their various courses of streptomycin over two and one-half years. In one, a consolidated area in the left mid-zone broke down and developed a cavity some weeks after the first course ceased. In spite of great temptation to collapse the lung, the attending physician was persuaded not to do so. The patient has now had six separate courses of treatment; her sputum has lessened or disappeared after each course and has temporarily become negative for tubercle bacilli. The cavity has steadily diminished in size, recession of the shadow being demonstrable by roentgenographic examination. After the first year, she showed no sign of ill health and put on considerable weight, her continued infection being a greater source of concern to her medical advisers than to her. At present, three years from the time of admission, her sputum is slight, watery, and free from tubercle bacilli six months after her last course of streptomycin and first course of PAS.

The other patient, a man of forty and an inveterate smoker not readily submitting to discipline, had shadows suggestive of "multiple small cavities in the right apex." Repeated courses of treatment, each after tubercle bacilli had reappeared in the sputum, appear to have maintained him in a state well enough to permit him to continue smoking and to return to work after the first sixteen months, and his sputum has been reduced to an almost neg-

ligible quantity. Roentgenographically, he has shown slow improvement for more than three years, and no culture positive for tubercle bacilli has been obtained during the past twelve months.

When present at all, the amount of sputum was not great in any case nor was it necessarily related to the extent of detectable cavitation. One patient with a hardly recognizable shadow (Case 37) was coughing up sputum with readily identified acid-fast bacilli in each field and confluent growth in cultures. Only two courses of streptomycin were required to rid her sputum of demonstrable tubercle bacilli, and it has remained free for more than three and one-half years

General Summary of Results

The differences between the two series at the end of two years can be summarized as follows:

Among the 18 control patients:

3 had not been followed sufficiently long to be reported on (Cases 1, 2, and 3).

Of the remaining 15:

5 still showed signs of active infection, namely, roentgenographic evidence of spread to the opposite side in 3 (Cases 6, 7, and 10); extension of the area involved in one (Case 16); cultures of sputum positive for *M. tuberculosis* in 3 (Cases 6, 11, and 16); 2 were not tested.

9, although not showing signs of activity, were still undergoing active treatment by collapse therapy.

One had discontinued collapse therapy, and the disease was considered to be quiescent (Case 8).

Among the 19 streptomycin-treated patients:

5 had not been followed for sufficiently long or had had collapse and PAS added to streptomycin treatment, invalidating their subsequent progress for the comparison (Cases 20 through 24).

Of the remaining 14:

4 still showed signs of active infection requiring further treatment, namely, roentgenographic deterioration on the same side in 2 patients and possibly in one other (Cases 29, 36, and 26); hemoptysis in one (Case 36); cultures of sputum positive for *M. tuberculosis* in 4 patients (Cases 26, 29, 30, and 36).

One started to discharge drug-resistant organisms within four weeks of starting treatment, and streptomycin was discontinued in favor of collapse (Case 19).

2 still required active treatment as a precautionary measure after parturition (Cases 32 and 33).

7 other patients were considered to have quiescent lesions. This conclusion was confirmed by subsequent follow-up for one year or more in 6 of the last 9 cases (Cases 30, 31, 33, 34, 35, and 37).

Disadvantages met during the conduct of the cases were as follows:

In "controls": Persistent fluid over the greater part of the two years was encountered in 4 patients; failure of collapse therapy to achieve its mechanical purpose was encountered in 2 patients; slower improvement in general health, as manifested by steady increase in weight and regaining of normal hemoglobin, was noted in comparison with streptomycin-treated cases.

In the streptomycin-treated cases: Relapses, roentgenographic or bacteriologic, occurred in 6 patients during the second year of the study. All of the 3 so treated were easily controlled by one or two courses of streptomycin alone. There was, however, no appearance of

fluid and no spread to the opposite side was seen. Both erythrocyte sedimentation rate and lymphocyte-monocyte ratio in active cases showed a more marked reaction when under the influence of streptomycin than in "controls." The significance of this phenomenon is not known.

DISCUSSION

Although the results reported here show many gaps and give indication of unnecessary changes of treatment, they may well be the only results which will ever be reported on the treatment of minimal tuberculosis by streptomycin alone and initial bed rest. Though the period of follow-up is short, it appears to be the best that can now be attained.

The method of treating the "control" series has not been critically appraised, for, although all workers may not agree that collapse therapy is always the ideal method of treating minimal lesions, it has great merits when minor and questionable cavities are so frequently found and rest in bed and full sanatorium care are not available to all. Moreover, its practice had become in this Unit a well-established routine and has the support of a prolonged study by Paine (12). The progress of these patients was simply regarded as a standard against which progress made following the newer therapy might be measured.

Comparison with the prognosis of active minimal lesions in Great Britain studied in the Prophit Survey (13), few of which were treated by collapse, indicates that both methods of treatment adopted in the investigation here reported have produced more favorable results than those followed for a comparable time in the Survey. Twenty-three of the 68 cases (34 per cent) in the Survey were found to have deteriorated roentgenographically within the first two years of observation. Five patients died, and 16 subsequently showed evidence of cavitation. In the present study, the 9 patients with progressing lesions (31.0 per cent) were not seriously ill and at no time appeared to be approaching death.

A particularly interesting finding in the streptomycin-treated series was the low incidence of the emergence of drug-resistant tubercle bacilli in the cases of minimal tuberculosis which were treated with streptomycin. Possibly this is characteristic of the type of lesion, as was suggested by Howlett, O'Connor, Sadusk, Swift, and Beardsley (3) who found that resistant tubercle bacilli appeared less frequently in lesions when caseation and cavities were absent than when they were present. Certainly, the one patient in whom highly resistant strains of tubercle bacilli emerged had a cavity and fluid, as did also the second who discharged bacilli which showed some lessening of drug sensitivity. No claim is made that the method of treatment accounts for this, but it was designed to detect any signs of emergence of resistant strains of *M. tuberculosis* before it had gone far, and was based on the original finding by Pyle (14). She cultivated on solid media successive samples of sputum from patients before, during, and after they had received courses of streptomycin. Thereby she detected in the samples of sputum a variety of organisms of different degrees of drug resistance and in different proportions, the proportion of resistant strains

usually growing as treatment continued. The same phenomenon was reported by Wolinsky, Reginster, and Steenken (18) who observed, further, that very occasionally in serial cultures, i.e., from 2 of their 145 patients, organisms reverted to full drug sensitivity three months after a fourth course of streptomycin had been discontinued.

It was this knowledge that led to the plan of treatment outlined here. It was thought that the more drug-sensitive tubercle bacilli, if still present and given sufficient time, might replace the more resistant ones (14, 18), and it was hoped that, after four weeks of treatment, it would be legitimate to withhold streptomycin for a sufficient length of time in a not very serious stage of tuberculosis for the possibly mounting proportion of drug-resistant strains to be outgrown again by sensitive strains, while the latter were not handicapped by the selective action of the drug. Streptomycin might then be reapplied when the preponderance of sensitive over resistant strains was at an optimum for therapeutic effect. The result seems to have been satisfactory except in one patient, and was unknown in 2 others whose treatment was altered after their organisms were found to be sensitive to only 5 to 10 γ per ml. Moreover, when the type of administration described in this study was adopted for 17 patients with more advanced pulmonary tuberculosis than those described in the present paper, the tubercle bacilli isolated were still sensitive to streptomycin after four weeks of treatment in 16, and in some patients after as many as three to five courses, the length of the intervening rest periods depending on the receipt of the results of drug-sensitivity tests.

While these observations were being made, sodium para-aminosalicylate became available for general use, and the problem of streptomycin resistance had consequently become much less serious. It was found, however, in the United States (17) and in Great Britain (7) that the addition of PAS reduced but did not abolish the phenomenon of the emergence of streptomycin-resistant strains of tubercle bacilli during treatment. The findings in the present report show that the onset of drug resistance might be reduced still further if minimal lesions were treated with chemotherapy and if each course were limited in duration.

It was a pity that the present observations, during which no more drug-resistant strains of tubercle bacilli appeared than might be expected even with the addition of PAS to the treatment, were terminated abruptly. Nevertheless, inasmuch as the results were not inferior to those obtained by the more "routine" procedure of collapse and bed rest, they should encourage more extensive work in which streptomycin is combined with PAS or with any other agent which may be found that reduces the incidence of the phenomenon of the emergence of drug-resistant strains of tubercle bacilli.

The work here described illustrates very well the difficulty of testing any new treatment of such a chronic disease as tuberculosis, as there are so many plausible and sometimes valid reasons for deviating from a regimen or procedure. Such deviation makes the value of deductions concerning the effects of any one therapeutic procedure almost impossible to assess. It must be borne in mind, however, as McCance (4) has remarked, that the physician "may not even

know that what he would have regarded as an 'unjustifiable experiment' five years ago may have become one of his standard diagnostic or therapeutic procedures."

The position of roentgenograms in the assessment of treatment and progress needs some enquiry. It has been studied intensively in the United States during the past few years. The difficulty of interpreting them has been emphasized by Medlar (8), whose study of 104 minimal lesions found at autopsy led him, as a pathologist, to state in 1950: "Neither is it possible to differentiate, by the character or roentgenographic shadows, between necrotic foci, non-necrotic inflammatory foci of long duration and scar formation." (9). In experienced hands, contradictory interpretations of progress have been obtained in as many as one-third of the cases whose films were examined before and after a three-month interval (19). This difficulty was fully recognized by the radiologists concerned, but so many serial roentgenograms were obtained that progress in one could be checked by the appearance in another until it seemed reasonable to assume that after two years a fair degree of accuracy in regard to deterioration or improvement might be relied on. Undoubtedly, roentgenograms have come to assume a position of prime importance in the routine application of collapse therapy, not only for the assessment of progress of the disease, but also for the more frequent assessment of the degree of collapse and for the presence or absence of adhesions or fluid, with the consequent indication for active measures of treatment. When these gross signs govern the mode of therapy, the bacteriologic report may be of secondary importance for, whether the sputum is positive or negative for tubercle bacilli, collapse will be continued for two years or more so long as it is producing the required mechanical effect as judged by roentgenographic findings.

When, however, a therapy is adopted which leaves the lung expanded, a very different criterion of effectiveness can be used. As long as a lesion communicates with a bronchus, microscopic detection of activity by the culture of *M. tuberculosis* from one of frequent serial laryngeal swabs, occasional sputum, or gastric washings would appear to be a much more delicate method of judging whether the lesion is becoming quiescent than serial roentgenograms over short intervals of time. Moreover, though without collapse the active focus may still be in communication with the exterior, after collapse of the lung, even when the lesion is still active, this may be prevented. This could be deduced from the early disappearance of cultures positive for tubercle bacilli from patients undergoing pneumothorax, and is supported by the microscopic observations of Medlar (9). The need for regular and frequent bacteriologic examinations during treatment thus rests on a reasonable foundation, as well as having been borne out in practice. From no fewer than 15 patients, tubercle bacilli were cultured after the first course of streptomycin, when few admitted to having any symptoms, and roentgenograms did not indicate progression of the disease in more than 4 of them.

The fluctuations in the size of roentgenographic shadows remarked on in the Prophit Survey were also a feature of the streptomycin series but, when they

were found to diminish regularly after the appropriate administration of the drug, their significance became of much less consequence.

Early second courses were given to all but 2 patients: one whose organisms were drug-resistant; and another, early in the series, whose progress in other respects at that time was considered satisfactory. Ten patients were given at least three courses of streptomycin; yet these courses, while apparently sufficient in the majority, were not adequate to control the infection in others, 2 of whom received as many as six separate courses of treatment. In spite of repeated courses no patient, with the exception of the one in whom the drug-resistant tubercle bacilli appeared early, failed to respond bacteriologically and clinically to each course of streptomycin given him.

The 4 patients described on pages 557, 558, and 559 in whom the bacteriologic warning was not heeded, show how the disease may progress if treatment is not instituted promptly. It cannot be too strongly emphasized that tuberculosis officers who perform refills with the utmost fidelity on patients undergoing collapse therapy at fortnightly intervals might equally faithfully arrange for a bacteriologic examination at similar intervals on patients being treated by chemotherapy. Undoubtedly, it would be useless to adopt streptomycin or other chemotherapeutic agents as a more routine form of therapy unless bacteriologic control had as prominent a place as has roentgenography at the present time.

SUMMARY

A series of 19 patients with minimal pulmonary tuberculosis treated with initial bed rest and streptomycin alone has been studied over a two-year period concurrently with a series of 18 patients treated with bed rest, 17 of whom also underwent collapse therapy. There was no unequivocal evidence that one treatment produced more rapid or certain control over the infection than the other, but there was reason to infer that chemotherapy could be used for minor lesions with a reasonable chance of success.

The main differences in favor of streptomycin were: no spread of infection to the opposite side, while spread of disease occurred in 3 patients who did not receive streptomycin; no persistent fluid in the pleural cavity, whereas fluid was present in 4 "control cases"; earlier signs of improved health, for example, gain in weight and in the concentration of hemoglobin; lessened amount of time spent at clinics and, for the doctor, less time spent in giving treatment.

Against streptomycin treatment was the early appearance of a highly drug-resistant strain of tubercle bacilli in one case and strains with reduced drug sensitivity in 2 others. All other strains of *M. tuberculosis* which persisted remained sensitive to 1 γ of streptomycin per ml. throughout therapy, which amounted in the aggregate to six months in some patients.

At the end of two years, in 7 of the 13 streptomycin-treated patients, whose prescribed treatment had been followed throughout, the lesions had reached quiescence. In one of 15 patients who received no streptomycin, the lesion was known to be quiescent. Five patients in each series had active lesions and the remainder were still undergoing collapse therapy, if controls, or were being observed, if in the

streptomycin-treated series, as the state of activity of their tuberculous lesions could not be fairly assessed.

SUMARIO

La Estreptomicina en la Tuberculosis Mínima

Una serie de 19 enfermos con tuberculosis pulmonar mínima tratada con reposo inicial en cama y estreptomicina sola ha sido estudiada durante un período de dos años juntamente con otra serie de 18 enfermos tratados con el encamamiento, y 17 de ellos además con la colapsoterapia. No hubo pruebas absolutas de que un tratamiento obtuviera dominio más rápido o seguro que el otro sobre la infección, pero sí hubo motivos para inferir que caba usar la quimioterapia para lesiones de menor orden con bastantes probabilidades de éxito.

Las principales diferencias en pro de la estreptomicina fueron: falta de propagación de la infección al lado opuesto, mientras que la dolencia se progagó en 3 enfermos que no recibieron estreptomicina; no hubo derrame persistente en la cavidad pleural, en tanto que lo hubo en 4 "casos testigos"; signos más tempranos de mejoría, por ej., aumento de peso y de la concentración de hemoglobina; menos tiempo pasado en las clínicas, y en lo tocante al médico, menos tiempo gastado en suministrar tratamiento.

En contra de la estreptomicinoterapia hubo la aparición temprana de una cepa sumamente farmacorresistente de bacilos tuberculosos en un caso y de cepas menos farmacosesibles en otros 2 casos. Todas las demás cepas del *M. tuberculosis* que persistieron continuaron siendo sensibles a 1 γ de estreptomicina por ml. durante toda la terapéutica, lo cual en algunos sujetos representó seis meses en conjunto.

Al cabo de dos años, en 7 de los 13 enfermos tratados con estreptomicina, en los que el tratamiento prescrito había sido observado constantemente, las lesiones habían alcanzado quiescencia. En uno de los 15 que no recibieron estreptomicina, se averiguó que la lesión estaba quiescente. Los demás, en ambas series, o bien tenían lesiones activas o, en la serie "testigo", recibían la colapsoterapia, de modo que no podía justipreciarse el estado de actividad de sus lesiones tuberculosas.

RESUME

La streptomycine dans la tuberculose avec lésions minimes

Une série de 19 malades, présentant des lésions pulmonaires minimes et soumis au repos au lit et à la streptomycine, exclusivement, a été étudiée pendant une période de deux années, en même temps qu'une autre série de 18 malades qui ont été traités par le repos au lit; 17 de ces malades étaient en outre soumis à la collapsothérapie. Des preuves indiscutables de la supériorité de l'efficacité et de la rapidité d'action d'un des traitements par rapport à l'autre n'ont pas été obtenues, mais il y a lieu de croire que la chimiothérapie pourrait être utilisée avec des chances de succès dans le traitement des lesions légeres.

Les différences principales en faveur de la streptomycine furent: l'absence d'extension de l'infection au côté opposé, tandis que l'infection s'est propagée chez 3 malades ne recevant pas de streptomycine; l'absence d'épanchement persistant dans la cavité pleurale, tandis que le liquide était présent dans 4 "cas témoins"; des signes précoces d'amélioration de l'état général, par exemple l'augmentation du poids et de la concentration de l'hémoglobine; une réduction de la durée de l'hospitalisation et, pour le médecin, une économie de temps dans le traitement.

Contre la streptomycinothérapie: l'apparition précoce d'une souche streptomycinorésistante de BK, dans un des cas, et de souches présentant une diminution de sensibilité au médicament, dans deux autres cas. Toutes les autres souches de *M. tuberculosis*, qui ont subsisté, sont demeurées sensibles à 1 γ de streptomycine par ml. pendant la durée du traitement, dont l'ensemble a atteint six mois chez certains malades.

A la fin des deux annés, pendant lesquelles le traitement prescrit avait été suivi, les lésions avaient été stabilisées chez 7 des 13 malades soumis à la streptomycinothérapie;

chez un des 15 malades ne recevant pas de streptomycine, la lésion est demeurée stable. Les autres sujets des deux groupes ou bien présentaient des lésions en activité ou, dans les séries "témoins", ils étaient soumis à la collapsothérapie, de telle sorte que le degré d'activité de leurs lésions tuberculeuses ne pouvait être déterminé.

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DETECTION OF SMALL NUMBERS OF TUBERCLE BACILLI FROM DISPERSED CULTURES, USING MICE, GUINEA PIGS, AND ARTIFICIAL MEDIA[1, 2]

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INTRODUCTION

Much has been written on the relative merits of three recognized technical procedures employed in obtaining bacteriologic confirmation of tuberculous infection in man, viz., smear, artificial cultivation, and animal inoculation.

While none will question the inferiority of the direct smear (which Corper (4) showed to be at least a thousand-fold less sensitive than the guinea pig), even when "choice particles" are selected, the procedure combines rapidity with the ability to detect all but the difficult minority of cases. It must, therefore, remain the most simple and practicable laboratory aid in the diagnosis of tuberculosis. However, in the "smear-negative" case with clinical or roentgenographic signs and the clinically resolved case in which a bacteriologic clearance is required, as well as in differentiating saprophytic acid-fast bacilli, the slower but more selective procedures must be employed. The evidence for and against each of the two remaining methods should be considered carefully before following the present trend to discard one in favor of the other.

Currently held views are, for the most part, based on examination of tuberculous exudates in routine diagnostic laboratories. Most of the comparisons made could be criticized, not as carefully conducted and recorded observations, but rather on the basis of the number of variables involved over which little control is possible and concerning which our knowledge is still inadequate. Prominent among these are the following:

(a) Wide variation in the numbers of viable bacilli originally present in the specimens; (b) loss of viability and of numbers during digestion which may be as high as 90 per cent of the original content, as observed by Freund and Middlebrook (11); (c) selective action of digesting agents, such as has been described by Bloch (2), adversely affecting the virulence of tubercle bacilli but not their ability to grow on media; (d) sampling errors involved both in the size of the sample taken respectively for smear, culture, and inoculation, and in the statistical scatter of organisms through the specimen; (e) the tendency of slow centrifugation speeds to select larger clumps of organisms, leaving single cells and smaller clumps in the supernatant fluid; and finally (f) the presence of contaminants not destroyed during digestion which may either overgrow cultures or in some cases result in the death of the animals.

Each of these variables presents a separate facet of the problem awaiting assessment. Meanwhile, different laboratories are employing available means of

[1] From the School of Bacteriology, University of Melbourne, Victoria, Australia.

[2] Assisted by grants from the Winter and the Haley Trust Funds.

minimizing their effects, often thereby departing further from circumstances which would indicate the true ability of animals and of media to detect small numbers of *Mycobacterium tuberculosis*.

The development of a simple semi-synthetic medium promoting dispersed growth by Dubos and Davis (7), and the adaptation of viable count methods to suit the growth requirements of *M. tuberculosis* by Fenner and others (9a, 9b), provided an opportunity to re-examine the question without the influence of at least some of the variables mentioned above. The necessity for a cheaper, more prolific, homozygous and rapidly maturing experimental animal than the guinea pig and one which would develop diagnostic lesions in a shorter period led us to consider the mouse as an alternative experimental animal.

Relatively little consideration appears to have been given to the response of the mouse to very small infecting doses of tubercle bacilli (13, 25); and it is obvious that, if the mouse is to be of use in diagnosis, it must possess tangible advantages over the guinea pig. The mouse must be at least as sensitive and present lesions readily recognized macroscopically and capable of confirmation by more specific means. Also the method of inoculation must be technically simple, not likely to produce a greater degree of dissemination of tubercle bacilli through the animal house than the infected guinea pig and, at the same time, the dose of concentrate administerable must not be too restricted.

Pierce and her co-workers (25) report that the most constant and typical lesion of experimental mouse tuberculosis, regardless of the portal of entry, is the grayish, circumscribed gelatinous tubercle occurring in the lungs. On the assumption that maximal concentration in the lungs of the bacilli present in the inoculum would be achieved by intranasal instillation, the present writers favored this route. Milzer and Levine (23) and Milzer (24) found the subcutaneous and intraperitoneal routes, previously shown to be relatively insensitive by Lange (19), and Pierce, Dubos, and Middlebrook (25), to be unsuitable even when mucin was added to the concentrates and, accordingly, they were not included in the present studies. Pierce and her co-workers (25) concluded that the intracerebral route was sensitive to tuberculous concentrates. It is not practicable, however, because of the susceptibility of the cerebral tissues to toxic factors in some concentrates, apparently independent of the presence of tubercle bacilli. The small volume admissible by this route is another contraindication to its use, but it was included in the present experiments to determine the sensitivity of mice to pure cultures via different portals of entry. The intravenous route has been adapted successfully to chemotherapy experiments by Youmans and Raleigh (28) and by McKee and co-workers (21). Lange (18), who made the first detailed studies of this route noted, however, that the results were variable, even with the same dose. Although the intravenous route seems to offer a less direct passage for tubercle bacilli to the lungs and, in the writers' opinion, is less readily performed than intranasal inoculation on the pigmented strain of mice which it was intended to use, the intravenous route was also included for comparison. As a preliminary step it appeared advisable to compare the relative susceptibility of approved cultural methods, guinea pig inoculation, and mouse

inoculation, using diminishing doses of organisms which had not previously been subjected to treatment which may or may not interfere with the ability of *M. tuberculosis* either to grow on artificial media or to infect experimental animals.

The present report deals with the results of the comparison.

EXPERIMENTAL METHODS

Media

As a precaution against contamination with fatty acids and other toxic substances, all glassware was acid-washed.

Tween[3]*-albumin fluid medium:* This medium, described by Dubos and Davis (7), has been used in this laboratory with success since that time for studies requiring dispersed growth of *Mycobacteria*.

Oleic-acid-albumin agar (OAA): This medium, devised by Dubos and recommended by Fenner and his co-workers (9a, 9b) has been used as the standard medium for viable unit counts. It has been found to be convenient and economical to use a two-layer plate with a saline-agar base onto which the OAA medium is poured to give a good depth of medium.

Modified Löwenstein medium: The preparation of this medium, which has been used for cultural examination of suspected tuberculous material in the Public Health Laboratory of Victoria since World War II, when certain ingredients normally included were unobtainable, is as follows:

Potassium di-hydrogen phosphate	3.2 gm.
Magnesium citrate	0.8 gm.
Ferrous sulfate	0.32 gm.
Bacto asparagine	4.8 gm.
Glycerol A.R.	48.0 ml.
Distilled water	800.0 ml.

These ingredients are steamed to dissolve (fifteen to twenty minutes); cooled to 50°C. or less, and 1.2 liters of well-beaten whole egg added. After addition of 20 ml. of a 5 per cent solution of malachite green, the medium is filtered through gauze, distributed in 6 ml. quantities in screw-capped bottles of 30.0 ml. capacity, and inspissated at 90°C. in a sloping position on three successive days. The initial period is for one hour; the others for twenty minutes.

Maintenance of Strain

Following recovery from an experimentally infected guinea pig, a reserve stock of lyophilized ampoules of strain H37Rv was prepared, using the apparatus devised by Gray (14). A fresh ampoule was opened after each eighth transfer so that the chances of alteration in virulence were minimized.

Inoculation Cabinet

The need to protect laboratory personnel from airborne organisms (27) led the present writers to carry out all manipulations behind glass and under ultraviolet light in a cabinet fitted with an exhaust fan. The discharged air passes through a glowing electric grid to destroy suspended organisms and then into a chimney communicating with the outside air. Masks are worn for added protection.

Experimental Animals

Mice: The mice used belong to the C57 black strain from the Roscoe Jackson Memorial Laboratory (introduced into Australia in 1947 by Rubbo and since maintained as a pure

[3] Proprietary name of the polyoxyethylene derivative of Sorbitan monooleate, Atlas Powder Co., Wilmington, Delaware.

line). Comparisons were made with multicolored mice from the Walter and Eliza Hall Institute strain, maintained by careful outbreeding since 1926, and with an albino strain from the writers' laboratory which has been inbred as a closed line for the past ten years. The average age at the time of infection was six weeks and the average weights 17 to 20 gm. for the C57 strain and 20 to 25 gm. for the other two breeds. The animals were fed on a balanced dry cereal mixture with vitaminized oil and green feed supplement. A note is made in the text in regard to those experiments in which, in view of the reports of Dubos and Pierce (8) and of Milzer and Levine (23) that animals appear more susceptible on a lower nutritional plane, changes were made to a deficient diet at the time of inoculation.

Guinea pigs: These animals, of a strain maintained as a closed line for many years, were fed on the same over-all diet as the mice, and any deviations have also been noted. Their average weight at inoculation was 300 gm. The period of observation is indicated in each experiment.

Counting Technique

It was obvious from the beginning that accurate viable-*cell* counts could not be obtained. As it was not desired to risk damaging the individual organisms to a degree which might influence their relative ability to grow *in vivo* and *in vitro*, grinding and the use of ballottini were avoided as means of obtaining greater dispersal. In view of this, it seems wise to follow the suggestion of Fenner (9) to refer to "viable units" rather than "viable cells." That these units are relatively stable to dilution procedures was shown by Fenner and largely confirmed by the present writers' own observations.

No attempt was made to determine the total bacterial count of the cultures. Having no means of determining whether individual organisms seen in a Helber chamber are living or dead, this appears to have little bearing on the problem, although the selection of twelve-day cultures possibly maintained a reasonably close ratio of total to viable numbers.

The "viable unit" count is based on the technique of Miles and Misra (22), with minor modifications by Gray (15). Standard (33 drop per ml.) dropping pipettes were used; these were rinsed five times in boiling water and cooled in sterile Ringer solution in preference to changing pipettes. Thus, the same pipette was used for making all the dilutions for the intranasal instillation of mice and for measuring the corresponding guinea pig doses, all of which were carried out consecutively. The diluent chosen was Dubos Tween-albumin medium.

The medium used for counting was oleic-acid-albumin agar with comparisons on modified Löwenstein medium. The plates were dried, open, in the incubator for two hours prior to sowing to ensure rapid absorption of the drops of inoculum and were finally sealed with clear cellulose tape to prevent evaporation. Colonies were counted after three weeks and checked after four weeks at 37°C., using an illuminated counting apparatus which gave 8 times magnification of the colonies.

Size of Inocula

Apart from the earlier experiments in which, because the response of the mice could not be anticipated, a wide range of inocula was adopted, an attempt was made to administer closely spaced doses of which the lower dilutions contained sufficient organisms to produce diagnostic lesions and the higher dilutions were free of infective units. This was not always successful, but the results obtained were usually clear cut, so this failure is perhaps of no great moment.

Because it was desired to have the dose suspended in a volume of 0.06 ml. (the mouse intranasal volume), dilutions of the culture were made as follows: After diluting the original culture 1 in 6, so that 0.06 ml. contained 0.01 ml. of original culture (10^{-2}), serial dilutions (usually tenfold) were then made and designated 10^{-3}, 10^{-4}, et cetera, representing that fraction of the number of organisms in 1 ml. of culture contained in 0.06 ml. of any given dilution. Thus, a mouse receiving the 10^{-6} dilution was given approximately one millionth of the viable units contained in 1 ml. of the culture. The counts were made from series of

single drops (0.03 ml.) of each dilution immediately before the animal infections were commenced. When mice and guinea pigs were inoculated with the same dilutions or when the intravenous route was used in mice, the volume was increased by diluting one-tenth to give an easily handled inoculum of 0.6 ml.

Route of Inoculation

Guinea pig: The standard route adopted in the Public Health Laboratory procedures was used, i.e., deep intramuscular inoculation in the left thigh.

Mice. Intranasal route: The method of Burnet and Timmins (3) was performed in the inoculation cabinet. The inoculum, suspended in a volume of 0.06 ml. (2 drops of a standard 33-drop pipette), was delivered onto the nostrils of the animals under ether-chloroform anesthesia. Any animal failing to inhale the inoculum completely and evenly was destroyed. As, in most instances, the experiments have required the administration of a number of different dilutions of culture, the practice was adopted of using the same pipette for any given series. Starting at the highest dilution, the inside of the pipette is thoroughly wetted with each dilution before instillation is commenced. The cages of inoculated animals were left in the cabinet under ultraviolet light for one hour until the estimated immediate danger period of return of organisms from the respiratory tract to the air was past (27). The risk of cross-infection was assessed by including suitably marked uninoculated controls with those animals receiving the largest intranasal dose.

Intravenous and intracerebral inoculations were carried out in the usual manner.

Recognition of Infection

The pathological picture of tuberculosis in guinea pigs inoculated intramuscularly needs no comment. In the mouse the obvious diagnostic lesion is the circumscribed gelatinous tubercle occurring in the lung. Contrary to expectations, tubercles are seldom invisibly located in the lung parenchyma and, when present, are readily detected.

It was found that while the lesions in the C57 strain tended to remain as discrete tubercles when moderate to small doses were given, and therefore were readily recognizable, with the multicolored and Albino strains a different pathology was presented. In the latter two, although initial infection with a given dose of small magnitude was less certain, the lesions, once established, tended to spread and coalesce, involving whole lobes in a general pneumonic consolidation which was less characteristic and, therefore, less readily differentiated with the naked eye from another pneumonic process which was encountered in one or two animals due to *Pasteurella pneumotropica* (16, 17). The yellowish abscesses seen in this condition which do not resemble the lesions produced by *M. tuberculosis* are not likely to be confused with them.

Microscopically, the findings in the present studies are in general agreement with those of Gerstl and Thomas (12), viz., that the microbial population of the tubercles varies from mouse to mouse and with the age of the lesion. At two weeks, with large doses, the field is usually packed with acid-fast bacilli but these diminish until at nine or ten weeks, or even earlier, bacilli are hard to find even by cultural methods. But, inasmuch as the mouse appears to have developed its diagnostic lesions within three weeks of inoculation, this is probably of no great importance diagnostically.[4]

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[4] Experiments now in progress to test the reliability of direct smear for confirming the tuberculous nature of typical lung lesions in mice suggest that this method may miss some cases when the inoculum is small. Following intranasal inoculation with Na_3PO_4 concentrates of sputum "negative" on smear (and occasionally by culture), it has been found that an appreciable proportion of typical lesions contain no acid-fast bacilli detectable with Ziehl-Neelsen staining at three weeks. Some of these results became positive with fluorescent staining, and an additional number, with culture or by confirmation from guinea pigs inoculated with the concentrate. Therefore, some rapid confirmatory test other than direct smear seems necessary. Whether the histopathologic changes observed on frozen sections are sufficiently constant for this purpose remains to be studied.

Cultural confirmation of the tuberculous nature of the lung lesions may be obtained by smearing the cut surface of the lesion onto horse-blood agar and oleic-acid-albumin agar plates, with and without added penicillin.

RESULTS

Comparison of Selected Routes of Inoculation

As a preliminary step, upon the result of which would depend subsequent experiments, it was necessary to demonstrate the most effective route for producing diagnostic lesions in the lungs and to ascertain the average number of organisms grown under the conditions described above which would give rise to a predictable degree of infection in a large proportion of mice.

With these objects in view, groups of five C57 mice were inoculated with three widely spaced dilutions of twelve-day cultures in Tween-albumin medium by each of the selected routes, viz., intranasal, intravenous, and intracerebral. All groups were maintained on full diet and the mice were well grown, weighing 20 to 23 gm.

TABLE 1

COMPARISON OF SELECTED ROUTES OF INOCULATION WITH M. TUBERCULOSIS H37Rv IN C57 MICE ON FULL DIET

DILUTION	VIABLE UNITS PER DOSE	PROPORTION SHOWING DIAGNOSTIC LESIONS AFTER 20 DAYS		
		Intranasal	Intravenous	Intracerebral
10^{-1}	Ca. 500,000	5/5	5/5	2/5
10^{-4}	500	5/5	2/5	0/3*
10^{-7}	0.5	1/5	0/5	0/5

* Two mice in this group died within twelve hours of inoculation.

One mouse in each group was sacrificed at fourteen days and examined for "diagnostic lesions." Smears were made from the lungs and other organs, and cultural confirmation was obtained by sowing selected tissues onto blood agar and OAA medium containing 100 units of penicillin per ml. The remaining animals were sacrificed on the twentieth day and examined principally for "diagnostic lesions" in the lungs. Table 1 shows the results of the over-all experiment.

Despite the wide spacing of dilutions used, the superiority of the intranasal over the other routes is indicated. Mice inoculated by the intracerebral method showed a numerical inferiority, in not only tendency to develop "diagnostic lesions," but also in the number of lung tubercles which developed. There were fewer tubercles in their lungs than in those of mice inoculated intranasally with a dose one thousand times smaller. The intravenous group occupied an intermediate position.

The distribution of lesions and of tubercle bacilli in those mice sacrificed at fourteen days was as follows:

The *intranasally infected mouse* which received 500,000 "units" showed hepatization of approximately 40 per cent of the lung tissue with many well-defined but closely approximated tubercles. Microscopically, the cut surface showed packed acid-fast bacilli in all

fields and yielded a pure, heavy culture of *M. tuberculosis*. The spleen was normal in size and appearance, no acid-fast bacilli were detected in a ten-minute search, and cultures produced only a few colonies, indicating little escape of inoculated organisms into the blood stream up to this stage. The counterpart mouse which received 500 "units" intranasally showed approximately 25 small but readily detected lung tubercles found to contain acid-fast bacilli, while spleen smear and culture were negative. The remaining mouse was negative on all counts.

The *intravenously infected mouse* (large dose) showed approximately 30 lung tubercles confirmed on smear and culture. The spleen, however, was grossly enlarged but not otherwise visibly abnormal. Large numbers of acid-fast bacilli were seen in a stained smear and confirmed as tubercle bacilli by culture. The smaller dose by this route caused no lung lesions, but the spleen was enlarged and infected while the remaining mouse was completely negative.

Intracerebral inoculation with the large dose failed to produce lung lesions detectable at fourteen days. The outstanding gross lesion was the enlarged spleen. Smear and culture revealed numerous bacilli in the brain and spleen. Lung smears were negative for acid-fast bacilli, but a few colonies appeared on culture. The smaller dose caused brain and spleen infection but had not reached the lungs, while the last animal appeared to have escaped infection. The picture on the twentieth day confirmed and extended these findings.

Taken in conjunction with the over-all finding, the intranasal route appeared to be the most satisfactory method of inducing lung lesions at both dose levels, but to bridge the wide gap between doses a second experiment was carried out, this time omitting the intracerebral route. Because concurrent experiments to be reported later seemed to confirm the findings of Dubos and Pierce and of Milzer and Levine that a lower plane of nutrition after infection induced greater susceptibility, the diet was reduced to water plus dried cereal pellets known to be inadequate alone for guinea pig nutrition. The upper limit of dose was planned to be in the vicinity of 5,000 units and tenfold dilutions followed. The mice were sacrificed at twenty-one days and the results appear in table 2.

At all dose levels from 800 units downwards, the animals in the intranasal groups show more numerous lesions. This result leaves no doubt of the superiority of the intranasal route and it has been used in all subsequent experiments. The results of viable unit counts carried out in this instance on oleic-acid–albumin agar medium gave the first indication that artificial media may be less sensitive than mice on a reduced diet to small doses of pure culture.

Susceptibility of C57 Mice to Other Acid-fast Bacilli

A small experiment was performed to study the reliability of the diagnostic lung lesion confirmed by smear alone. Twelve-day cultures of the BCG strain and of H37Rv in Tween-albumin medium and a three-day culture of a laboratory strain of *M. phlei* were used. The H37Rv culture was killed by heating to 60°C. for one hour. Then all three cultures were brought to a uniform turbidity, using an Evelyn photoelectric colorimeter, galvanometer reading 36 with a 660 filter. This dilution, a heavy cell suspension, together with another which was one

hundredfold less dense, was instilled intranasally into groups of four mice. When sacrificed at three weeks, all mice receiving the larger dose of BCG showed numerous scattered tubercles indistinguishable macroscopically and microscopically from those due to the virulent strain. The smaller dose of BCG and the other inocula failed to produce any lung lesions.

Comparison of the Susceptibility of Mouse Strains on Restricted Diet to Intranasal Inoculation

Of the several mouse strains known to be highly susceptible to experimental tuberculosis, only one is available in this country. The C57 black strain was compared with multicolored mice from the Walter and Eliza Hall Institute and the albino strain from the present writers' laboratory. Though of the same age, the more slowly maturing C57 mice were 5 to 7 gm. lighter than the other two.

TABLE 2

COMPARISON OF INTRANASAL AND INTRAVENOUS INOCULATION WITH M. TUBERCULOSIS H37Rv IN C57 MICE ON REDUCED DIET

CULTURE DILUTION	VIABLE UNITS	INCIDENCE OF DIAGNOSTIC LUNG LESIONS			
		Intranasal		Intravenous	
		Number	Degree	Number	Degree
10^{-4}	Ca. 8,000	6/6	++++	5/5	+++
10^{-5}	Ca. 800	6/6	+++	2/6	+
10^{-6}	78	6/6	++	0/6	−
10^{-7}	6	4/6	+	2/6	+
10^{-8}	0.7	4/6	+	2/6	+
$10^{-8.5}$	0	2/6	+	0/6	−
10^{-9}	0	1/6	+	0/6	−

++++ = > 30 tubercles; +++ = 20 to 30 tubercles; ++ = 10 to 20 tubercles; + = 1 to 9 tubercles.

The diet was restricted at the time of inoculation with two moderately heavy doses, and the mice were sacrificed in two groups at intervals of two and six weeks (table 3).

After two weeks the tuberculous lesions were well developed; the C57 animals which received approximately 140 viable units showed diagnostic lesions in 3 of 5 cases. The C57 strain showed a marked advantage over the multicolored strain in the number of lesions which developed, with the albinos in an intermediate position. After six weeks these differences tended to flatten out, the latter two strains showing generally far more extensive consolidation of lung tissue than the C57 strain in which tubercles tended to enlarge slowly rather than to coalesce.

Therefore, although individual animals of the Australian strains showed a greater tendency to extensive lung involvement once they had become infected, a tendency which was marked at both dose levels, it was less certain that a given dose would produce a predictable number of lesions.

The Rate of Development of Diagnostic Lesions in C57 Mice and Guinea Pigs on Full Diet

While readily detectable lesions apparently can be expected within ten to fourteen days of the intranasal inoculation of large doses of tubercle bacilli, it was necessary to determine the minimal period required to establish lung lesions with

TABLE 3

COMPARISON OF THE SUSCEPTIBILITY OF DIFFERENT MOUSE STRAINS TO INTRANASAL INOCULATION WITH M. TUBERCULOSIS H37Rv (RESTRICTED DIET)

STRAIN	VIABLE UNITS (AVERAGE 6 COUNTS)	2 WEEKS		6 WEEKS	
		D. L.	Degree	D.L.	Degree
C57	Ca. 1,400	5/5	Tubercles ++++	5/5	Tubercles ++++
Multicolored	Ca. 1,400	3/5	Tubercles +	5/5	Extensive general consolidation
Albino	Ca. 1,400	4/5	Tubercles ++	5/5	Extensive general consolidation
C57	140	3/5	Tubercles ++	5/5	Tubercles ++++ small
Multicolored	140	0/5	—	4/5	Tubercles ++ large
Albino	140	1/5	Tubercles +	5/5	Coalescing lesions

D. L. = diagnostic lesions; + = 1 to 5 tubercles; ++ = 5 to 10 tubercles; +++ = 10 to 30 tubercles; ++++ = > 30 tubercles.

TABLE 4

RATE OF DEVELOPMENT OF DIAGNOSTIC LESIONS IN C57 MICE AND IN GUINEA PIGS INOCULATED WITH M. TUBERCULOSIS H37Rv (FULL DIET)

DILUTION	VIABLE UNITS (AVERAGE 8 COUNTS)	2 WEEKS		3 WEEKS		4 WEEKS	6 WEEKS	
		Mice	Guinea Pigs*	Mice	Guinea Pigs	Mice	Mice	Guinea Pigs
10^{-4}	Ca. 1,600	5/5	—	5/5	4/4	5/5	5/5	4/4
$10^{-4.5}$	300	5/5	—	5/5	4/4	4/4	5/5	4/4
10^{-5}	160	0/5	—	5/5	3/4	5/5	5/5	4/4
10^{-6}	14	0/5	—	5/5	0/4	5/5	5/5	4/4
10^{-7}	0.5	0/5	—	4/5	0/4	4/5	4/5	4/4
10^{-8}	0	0/5	—	2/5	0/4	3/5	3/5	4/4

* As no palpable lesions had developed either at the inoculation site or in the superficial inguinal lymph nodes at two weeks, these animals were not sacrificed.

very small inocula. In the guinea pig this is generally considered to vary from six to eight weeks or longer, according to accepted practice.

Groups of twenty mice were inoculated with doses ranging from 10^{-5} to 10^{-8}. As circumstances necessitated restricting the number of guinea pigs, it was decided not to sacrifice any of those available until palpable lesions had developed in the larger dosage groups.

Both groups of animals were maintained on full diet. The mice were sacrificed after two, three, four, and six weeks in groups of five and the guinea pigs at the third and sixth week in groups of four (table 4).

The results were assessed in terms of "diagnostic lesions" in both instances. Inferences may be drawn from these results relating to the rate of development of diagnostic lesions in both species, to the relative susceptibility of mice and guinea pigs on full diet to small doses of tubercle bacilli, and to the selectivity of animals compared with oleic-acid–albumin agar medium. Doses of 300 or more "viable units" resulted in "diagnostic lesions" in all mice by the fourteenth day; with smaller doses, however, the response was slower, although in all animals in which infection did eventually become established it was evident macroscopically by the twenty-first day. At three weeks, only those guinea pigs which received 160 or more "viable units" had developed caseating lesions which, however, had not progressed along the lymphatic chain beyond the inguinal and/or precrural nodes. In a few animals the sublumbar nodes were enlarged and hemorrhagic but showed no visible caseation. These observations leave no doubt that the recognizable mouse response to experimental tuberculosis is a good deal more rapid than that of the guinea pig. At six weeks, however, the total score of guinea pigs, especially at the lower dose levels, is rather better than that of the mice. Later experiments relate this to the level of nutrition.

It will be observed that both species appeared to be more sensitive to small inocula than did oleic-acid–albumin agar medium, infection occurring in both at a tenfold higher dilution than the last to give a positive culture and, had more groups been included, the observed differences may even have exceeded this figure. As the drops (0.03 ml.) counted for each dilution numbered eight, a total equivalent to four animal doses, it cannot be argued that sampling error alone was responsible, especially in view of later repetition of these results. It is of interest to record, however, that in the 10^{-7} dilution, the 8 drops counted yielded only four colonies, of which two were in one drop.

The Relative Efficiency of Media, Mice, and Guinea Pigs (Diet Restricted)

Additional confirmation of the relative efficiency of the mouse, of the guinea pig, and of routine cultural methods in detecting small numbers of H37Rv seemed necessary in view of this rather unexpected trend of results. The dilutions used were extended further with closer spacing and with Löwenstein and Dorset egg media included as controls on oleic-acid–albumin agar. Moreover, the rather erratic distribution of viable units over replicate doses of the higher dilutions suggested the necessity to examine its influence on the chance that an individual animal in a given group would or would not receive an infective dose. Although Löwenstein's medium made up in plates proved satisfactory, Dorset's egg medium dried out even though the plates were carefully sealed, restricting the comparison to two media (table 5).

The mice were sacrificed at three weeks and the guinea pigs at six weeks to allow for the slower development of lesions in these animals. The diet of both mice and guinea pigs was reduced at the time of inoculation as before. The nutritional status of the mice did not appear to be greatly affected, but the diet was quite inadequate to sustain guinea pigs. By the fourth week all the latter animals were extremely emaciated and three died of starvation within three days of completing the experiment. Two of these, in groups receiving small doses, showed

no lesions of tuberculosis and there was no evidence that the reduced diet increased susceptibility to infection as it appeared to do in the mice. The findings reveal several important facts. There is, for example, an unusually close agreement, in view of the expected margin of error, between the counts on the two media and also in the actual and anticipated dilution decrease. This confirms the reports of Fenner and his co-workers (9a, 9b) of the ability of the semi-synthetic oleic-acid–albumin agar to promote the growth of small inocula and shows it to be the equal of the routine diagnostic medium. The failure of certain animals in each group among the higher dilutions to develop lesions is no doubt explicable in part by the observed irregular distribution of viable units in the inocula.

TABLE 5

RELATIVE EFFICIENCY OF MEDIA, MICE, AND GUINEA PIGS IN DETECTING M. TUBERCULOSIS H37Rv (DIET RESTRICTED)

DILUTION	VIABLE UNITS PER DOSE AVERAGE OF 12 COUNTS		DIAGNOSTIC LESIONS	
	Oleic-Acid-Albumin Agar	Löwenstein	Mice (3 weeks)	Guinea Pigs (6 weeks)
10^{-6}	Ca. 3,000	Ca. 3,000	25/25	15/15
$10^{-6.5}$	280	256	25/25	15/15
10^{-7}	23	27	20/25	12/15
$10^{-7.5}$	4	5.6	16/25	10/15
10^{-8}	3.4	2.8	16/25	6/15
$10^{-8.5}$	0.8	0.5	11/25	4/15
10^{-9}	0	0	14/25	3/15
$10^{-9.5}$	0	0	5/25	0/15

Distribution of viable units in higher dilutions shown by counts:

Oleic-acid-albumin agar: 10^{-7} = 3,0,3,2,0,3,2,0,1,2,3,2
= 21/12 = 1.68 per drop = 3.36 per dose

$10^{-7.5}$ = 1,0,0,0,1,1,0,0,1,1,0,0
= 5/12 = 0.42 per drop = 0.84 per dose

Löwenstein: 10^{-7} = 1,3,0,3,1,0,3,0,3,1,0,2
= 17/12 = 1.41 per drop = 2.82 per dose

$10^{-7.5}$ = 0,0,0,0,0,1,0,1,0,1,0,0
= 3/12 = 0.25 per drop = 0.5 per dose

A repetition of the previous experiment, designed to confirm the influence of diet on mouse susceptibility is summarized in table 6. As may be seen in the table, the advantage here appears to rest with the mice, although the numbers are small and the guinea pigs were sacrificed at four weeks.

From this and the previous experiment it appears a justifiable assumption that mice on a reduced diet are of the same order of susceptibility as guinea pigs but are more regularly infected by small doses of tubercle bacilli and develop diagnostic lesions more rapidly than do guinea pigs. Moreover, both mice and guinea pigs are more susceptible to small numbers of tubercle bacilli (H37Rv) grown for twelve days in Tween-albumin medium than to comparable small numbers which have been grown on the writers' modification of Löwenstein's medium or oleic-acid–albumin agar.

Epidemiological Considerations

It is intended to report separately on the dangers associated with the handling of tubercle bacilli but, as the anticipated reaction to the suggested use of intranasal inoculation is likely to be that the risk is unduly great (the procedure was eliminated by Donovick and his co-workers (6) on these grounds), it is necessary to mention briefly in the present text preliminary experiments in this connection.

TABLE 6

THE INFLUENCE OF REDUCED DIET ON MOUSE AND GUINEA PIG SUSCEPTIBILITY

DILUTION	VIABLE UNITS (AVERAGE 6 COUNTS)	DIAGNOSTIC LESIONS—4 WEEKS	
		Mice	Guinea Pigs
10^{-6}	Ca. 400	10/10	10/10
10^{-6}	39	10/10	9/10
$1/4 \times 10^{-6}$	7.8	8/10	7/10
$1/16 \times 10^{-6}$	2.1	5/10	5/10
$1/64 \times 10^{-6}$	0.7	5/10	2/10
$1/128 \times 10^{-6}$	0	4/10	1/10
$1/256 \times 10^{-6}$	0	3/10	0/10

The dilutions used were closely spaced and the guinea pigs observed for four weeks only.

TABLE 7

CROSS-INFECTION IN EXPERIMENTAL TUBERCULOSIS. RESULTS OF EXPOSING HEALTHY C57 MICE TO INFECTED MICE AND GUINEA PIGS

NATURE OF EXPOSURE TO WHICH NORMAL MICE WERE SUBJECTED	DURATION OF CONTACT	PROPORTION OF CONTACT MICE INFECTED AFTER 9 WEEKS	
		Number	Degree
Guinea pig. Ulcerated lesion following subcutaneous inoculation with sputum	4 weeks	3/5	+++
Guinea pig. Closed lesion following intramuscular inoculation with sputum	4 weeks	1/5	+
C57 mice IXIO[6] H37Rv. Intranasally	3 weeks	3/5	+
C57 mice IXIO[6] H37Rv. Intracerebrally	3 weeks	0/5	–
C57 mice 1,400 H37Rv Intranasally	2 weeks	1/5	+++
Albino mice 1,400 H37Rv. Intranasally	2 weeks	1/5	++
Multicolored mice 1,400 H37Rv. Intranasally	2 weeks	1/5	+++

Degree: +++ = 5 to 10 tubercles; ++ = 3 to 5 tubercles; + = 1 to 2 tubercles.

It is evident that any laboratory manipulation of materials containing living tubercle bacilli invariably involves a considerable degree of risk, but through common usage some procedures have come to be regarded as less dangerous than others. Practical precautions have been suggested by Fish and Spendlove (10) and by Anderson (1).

To test the risk involved in an empirical manner, healthy mice were placed in the same cage with animals receiving the largest dose of tubercle bacilli by different routes in a number of experiments. The contact commenced during inocu-

lation. Similar groups of healthy mice were placed in contact with guinea pigs which had been infected by intramuscular and by subcutaneous injection of sputum positive for *M. tuberculosis*. The contact animals were examined after nine weeks for typical lung lesions[5] (table 7).

It will be noted in table 7 that some degree of cross-infection occurred in practically every group. These preliminary experiments indicate that the danger of dissemination of tubercle bacilli in the animal house, though real, is probably no greater following intranasal inoculation of mice than from guinea pigs inoculated in the usual manner with tuberculous concentrates.

The risk to the operator during any manipulations involving living tubercle bacilli is such that it is considered unsafe to perform even the simpler procedures except in adequately fitted cabinets and unless properly masked and otherwise protected. Therefore, it is concluded at this stage that intranasal inoculation, properly performed, is not likely to increase the already present risk to the operators and attendants.

DISCUSSION

The results reported herein lead the writers to assume that the widely accepted view that cultural methods are more sensitive than guinea pig inoculation for the diagnostic isolation of tubercle bacilli and should supplant it is not a true reflection of the relative sensitivity of these two laboratory aids to normal virulent tubercle bacilli. On the contrary, it would seem that, when tubercle bacilli are not subjected to the probably selective and certainly lethal action of various chemicals used in the homogenization of sputum or other tuberculous exudates and to centrifugation at speeds which favor the recovery of clumps rather than single organisms, the reverse of this statement might well be true.

It is obvious that concentration processes act adversely on the tubercle bacilli present in the specimen, destroying as many as 90 per cent and losing others in discarded supernatant fluids. Moreover, the procedures allow the survival of a proportion of cells which are potentially abnormal in showing a high resistance to the chemicals employed and are so altered as a result of treatment that they may, as Bloch (2) suggests, be capable of reproducing on artificial media but not of infecting guinea pigs.

The present experiments suggest that, when not subjected to digestive processes, tubercle bacilli grown in Tween-albumin medium regularly cause infection in a proportion of animals at dilutions tenfold or more beyond the lower limit of cultural sensitivity. It should not be overlooked that bacilli so cultured are not "normal" in so far as they are grown in the presence of Tween 80. This is known to increase markedly their sensitivity to penicillin, but this abnormality does not appear to persist long enough after transfer of the organisms to an environment free of Tween 80 to cause decreased resistance to the defense mechanisms of the experimental animal. It is hard to imagine how cultural

[5] Smears from these lesions showed no acid-fast bacilli, a finding which is in keeping with the probable age of the lesions; and, as no contaminants were isolated, it is reasonable to assume that the lesions were tuberculous.

methods could be more sensitive than guinea pig inoculation if the careful experiments conducted by Doerr and Gold (5) are valid. They succeeded in causing tuberculosis in 10 per cent of 30 animals inoculated with one organism picked out with a micro-manipulator from 10- to 31-day cultures. Other workers using less sensitive counting methods, e.g., Lange (19), 1 to 5 organisms; Glover (13), 5 to 50 organisms; and Schwabacher and Wilson (26), more than 10 living bacilli, confirm the sensitivity of the guinea pig to very small inocula. Finally, the results of the present experiments with C57 black mice inoculated intranasally, as well as the experiments with guinea pigs, indicate that these animals are very sensitive to experimental infection. In fact, under the conditions of the present experiments, the mouse appeared more regularly susceptible to small doses of tubercle bacilli than did the guinea pig.

In seeking an explanation of the inferiority of the media used in the present study, the writers are reminded of the observation of Lurie and his co-workers (20) that aerosols derived from suspensions containing isolated bacilli were approximately ten times more tuberculogenic for rabbits than those derived from suspensions containing larger bacillary groups. It is probable that, although unfiltered, the suspensions contained in the higher dilutions mainly dispersed cells rather than clumps. Although Fenner and his co-workers, studying the growth of Tween-albumin cultures on oleic-acid–albumin agar, considered that satisfactory growth could be obtained from single cells, it is not impossible that larger clumps are required to initiate growth regularly on solid medium; thereby accounting for the observed differences between animals and media in the present experiments.

The position at the present stage of our knowledge would appear to be that less wasteful and less lethal concentration methods should be sought and the sensitivity of animals and culture media reconsidered under the optimal practical conditions which can be applied to routine diagnosis.

Encouraged by the rapid appearance of diagnostic lesions in mice infected intranasally, the writers propose to test their suitability for detecting tubercle bacilli in sputum in numbers not disclosed by routine examination of smears, in parallel with the existing culture technique. Experiments are now in progress, testing serial dilutions of sputum concentrates weakly positive for acid-fast bacilli by smear. At the same time, the lethal action of currently adopted and other concentration methods is under review.

SUMMARY

Current views concerning the relative value of culture media and of animals for the detection of tubercle bacilli are based on the response to selected organisms (the survivors of concentration methods) and are, therefore, suspect.

Both C57 mice and guinea pigs in the present experiments have proved to be at least ten times more sensitive than either modified Löwenstein or oleic-acid–albumin agar to H37Rv grown in Tween-albumin medium.

When inoculated intranasally and maintained on a low plane of nutrition, C57 mice appear to be a little more regularly susceptible to threshold doses than

do guinea pigs by the intramuscular route. By other routes of inoculation the mice were less susceptible when the results were assessed in terms of macroscopic lung lesions.

The appearance of diagnostic lesions is earlier in C57 mice on a reduced diet than in guinea pigs.

When carried out with the care considered necessary for any manipulations involving virulent tubercle bacilli, intranasal inoculation appears to be no more dangerous than other techniques.

SUMARIO

Descubrimiento de Pequeñas Cantidades de Bacilos Tuberculosos en Cultivos Dispersos, Usando Ratones, Cobayos y Medios Artificiales

Las opiniones reinantes acerca del relativo valor de los medios de cultivo y de los animales para el descubrimiento de bacilos tuberculosos se basan en la respuesta a microbios seleccionados (los sobrevivientes de técnicas de concentración), y por lo tanto, se hallan en tela de jucio.

Tanto los ratones C57 cuanto los cobayos usados en estos experimentos se mostraron a lo menos diez veces más sensibles que bien el medio de Löwenstein modificado o el agar-albúmina-ácido oleico, al H37Rv cultivado en medio de Tween-albúmina.

Inoculados intranasalmente y mantenidos en una esfera baja de nutrición, los ratones C57 parecen ser un poco más susceptibles regularmente a dosis límites que los cobayos inoculados por vía intramuscular. Por otras vías, los ratones fueron menos susceptibles cuando se apreciaron los resultados en términos de lesiones macroscópicas del pulmón.

La aparición de lesiones diagnósticas es más temprana en los ratones C57 a dieta baja que en los cobayos.

Llevada a cabo con el cuidado considerado necesario para toda manipulación en que figuran bacilos tuberculosos virulentos, la inoculación intranasal no parece ser más peligrosa que otras técnicas.

RESUME

Détection de bacilles tuberculeux en petit nombre dans les cultures à croissance dispersée, en utilisant la souris, le cobaye et un milieu artificiel

Les idées courantes, concernant la valeur relative des milieux de culture et de l'animal pour la détection du bacille tuberculeux, sont fondées sur la réponse de germes sélectionnés (les rescapés des méthodes de concentration) et qui, en conséquence, sont suspects.

La souris C57 et le cobaye soumis à ces expérimentations se sont, l'un et l'autre, montrés au moins dix fois plus sensibles que le milieu de Lowenstein modifié ou le milieu albumine agar-acide oléique pour le H37Rv cultivé dans le milieu Tween-albumine.

Inoculée par voie intranasale et maintenue à un niveau de nutrition bas, la souris C57 semble être un peu plus régulièrement sensible aux doses de seuil, que ne l'est le cobaye inoculé par voie intramusculaire. Lorsque d'autres voies d'inoculation ont été utilisées, la sensibilité de la souris a été plus faible quand les résultats ont été évalués en termes de lésions pulmonaires macroscopiques.

L'apparition de lésions diagnostiques est plus précoce chez la souris C57, soumise à un régime alimentaire réduit, que chez le cobaye.

Quand elle est effectuée avec le soin considéré nécessaire pour toute manipulation où interviennent des bacilles tuberculeux virulents, l'inoculation intranasale ne semble pas plus dangereuse que les autres techniques.

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THE EFFECT OF PNEUMOPERITONEUM THERAPY IN PULMONARY TUBERCULOSIS ON LIVER FUNCTION[1, 2]

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INTRODUCTION

During the past decade there has been increasing use of pneumoperitoneum in the treatment of pulmonary tuberculosis, either as a definitive procedure alone or in combination with antimicrobial and surgical forms of therapy. The factors which encouraged the wider use of pneumoperitoneum as a therapeutic procedure were: the simplicity of induction, its reversibility, the minimum of complications incident to its use, and the maintenance of the integrity of the intrapleural space.

The efficacy of pneumoperitoneum in the treatment of pulmonary tuberculosis has been well established by the work of Banyai (1), Trimble and Wardrip (2), and Edlin and Bassin (3).

Pneumoperitoneum has also been studied in the treatment of chronic pulmonary emphysema. Gaensler and Carter (4) and Furman and Callaway (5) have shown that the mechanical elevation of the diaphragm improved its function, resulted in a decrease in the residual air, increased vital capacity, and improved blood oxygenation.

Pneumoperitoneum in the treatment of pulmonary tuberculosis is a procedure which requires prolonged use. Frequently it is necessary to maintain refills for several years to achieve proper control and stability of the pulmonary disease. A variable increase in intra-abdominal pressure is, therefore, persistently present for many months or years. The consequences of this increase in pressure can be best appreciated by reviewing roentgenograms of the abdomen of patients under treatment with pneumoperitoneum. There is considerable separation and displacement of the liver from the diaphragmatic leaves, lengthening of the esophagus, compression of the abdominal viscera, and displacement of these towards the inferomedial plane.

The present study was done to determine the effect of continuous pneumoperitoneum therapy and its associated elevated intra-abdominal pressure upon liver function as determined by multiple liver function tests.

PLAN OF INVESTIGATION

Selection and analysis of cases: Twenty-five patients with pulmonary tuberculosis treated with pneumoperitoneum were carefully selected for study. The

[1] From the Medical Service, Veterans Administration Hospital, Brooklyn, New York.

[2] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

patients were all less than 35 years of age, in good physical and nutritional status, and free of demonstrable extrapulmonary tuberculous lesions. The pulmonary disease in each case was in the inactive state at the time that the liver function tests were performed. All patients had attained sufficient benefit from the pneumoperitoneum therapy to permit their discharge from active hospital care.

Patients with signs of uncontrolled or progressive pulmonary disease, history of alcoholism, malnutrition, or previous history of jaundice, hepatitis, or hepatomegaly were excluded from the study. Emphasis was placed on the exclusion of all possible factors having the potentiality of causing liver function derangement. The present study is, therefore, an analysis of the effect of pneumoperitoneum and long-continued intra-abdominal positive pressure, per se, on liver function.

Pneumoperitoneum technique: The pneumoperitoneum was induced by the conventional procedure. Sufficient air was injected at weekly or biweekly intervals to maintain a rise in the diaphragmatic leaves at the level of the fourth rib or intercostal space anteriorly as determined by frequent fluoroscopic and roentgenographic examinations.

Clinical and laboratory data for the 25 patients: In table 1 are summarized the essential clinical features in the 25 cases at the time of study of liver function relative to age, race, type and extent of the initial pulmonary lesion, duration of the disease, duration of the pneumoperitoneum, average intra-abdominal pressure, sputum or gastric content bacteriologic findings, antimicrobial therapy administered, and erythrocyte sedimentation rate. In the interpretation of the extent and activity of the pulmonary disease, the criteria in the 1950 edition of *Diagnostic Standards and Classification of Tuberculosis* of the National Tuberculosis Association were followed.

Twenty-two patients were white and 3 were non-white. The ages varied from twenty to thirty years in 16, and from thirty-one to thirty-four in 9 cases. The initial pulmonary disease was moderately advanced in 20 and far advanced in 5 instances. Single or multiple cavities were originally present in all cases. The duration of time from the induction of pneumoperitoneum to the final liver function studies varied from six to twenty-one months. The distribution of patients with relation to the duration of the pneumoperitoneum was as follows:

Duration of pneumoperitoneum (in months)	4 to 8	9 to 13	14 to 18	19 to 23
Number of patients	6	9	8	2

Thus, in 19 cases the duration of the pneumoperitoneum was nine months or longer at the time the liver function studies were conducted.

Average intra-abdominal pressure: The average intra-abdominal pressure varied from plus 8 to plus 20 cm. of water. In approximately one-half of the cases, the average intra-abdominal pressure was plus 12 cm. of water or higher throughout the maintenance of the pneumoperitoneum therapy. Four patients had a supplementary temporary phrenic crush. In each case the pulmonary disease was stable and inactive. The sputum or gastric contents were repeatedly negative for tubercle bacilli for periods varying from six to fourteen months. The duration of the pulmonary disease varied from nine to sixty months. In 21 cases the disease was of one or more years' duration. The erythrocyte sedimentation rates were within normal limits in all instances with the exception of Cases 9 and 11 who showed a rate of 17 and 19 mm. per hour, respectively.

All patients with the exception of one (Case 1) were treated with streptomycin and para-aminosalicylic acid (PAS) for varying periods during their course of hospitalization. In order to eliminate the possible hepatotoxic effects of streptomycin and PAS, the liver function studies were done after these medications had been discontinued for a period

TABLE 1

ESSENTIAL CLINICAL FEATURES IN CASES OF PNEUMOPERITONEUM IN THIS SERIES

CASE NUMBER	AGE	RACE	TYPE OF DISEASE	DURATION OF DISEASE	DURATION OF PNEUMOPERITONEUM	AVERAGE INTRA-ABDOMINAL PRESSURE CM. WATER	SPUTUM OR GASTRIC BACTERIOLOGY	STREPTOMYCIN THERAPY	PAS	MONTHS BETWEEN SM-PAS THERAPY AND LIVER FUNCTION TESTS		E.S.R.
										SM	PAS	
	years			*months*	*months*			*gm.*				*mm/hr.*
1	21	White	Moderately advanced, right upper lung	12	10	+10	Negative 8 months	—	—	—	—	11
2	34	White	Moderately advanced, right upper lung	12	8	+10	Negative 7 months	120	+	8	8	8
3	31	White	Far advanced, right upper lung and left upper lung	23	21	+15	Negative 12 months	150	+	13	2	1
4	29	White	Far advanced, right upper lung and left upper lung	29	20	+ 7	Negative 12 months	90	+	19	3	7
5	21	White	Moderately advanced, left upper lung	10	7	+ 8	Negative 6 months	45	+	6	6	5
6	24	White	Moderately advanced, right upper lung	12	8	+20	Negative 8 months	120	+	6	3	1
7	32	White	Moderately advanced, right lower lung	9	6	+ 8	Negative 6 months	150	+	Continued		1
8	28	White	Far advanced, right upper lung	12	9	+10	Negative 8 months	180	+	Continued		3
9	20	White	Moderately advanced, right upper lung	11	9	+ 8	Negative 6 months	40	+	8	8	19
10	36	White	Moderately advanced, right lower lung	20	8	+12	Negative 11 months	35	+	8	8	4
11	34	Non-white	Moderately advanced, right upper lung and left upper lung	60	15	+14	Negative 12 months	22	+	18	12	17
12	24	White	Moderately advanced, right upper lung	32	16	+ 8	Negative 14 months	60	+	25	25	3
13	34	White	Moderately advanced, right upper lung	16	12	+10	Negative 10 months	80	+	9	9	8
14	28	White	Moderately advanced, left upper lung	20	15	+12	Negative 8 months	100	+	15	15	10
15	26	White	Far advanced, left upper lung	14	12	+15	Negative 7 months	140	+	8	8	4
16	32	Non-white	Far advanced, left upper lung	22	16	+12	Negative 9 months	120	+	6	6	5
17	28	White	Moderately advanced, left lower lung	15	12	+ 8	Negative 10 months	140	+	5	5	9
18	25	White	Moderately advanced, right upper lung	20	14	+14	Negative 12 months	180	+	8	8	3
19	25	White	Moderately advanced, right lower lung	17	14	+18	Negative 7 months	120	+	9	4	12
20	34	White	Moderately advanced, left upper lung	15	10	+12	Negative 6 months	120	+	10	10	8
21	22	White	Moderately advanced, left upper lung	18	12	+14	Negative 8 months	140	+	12	6	7
22	28	Non-white	Moderately advanced, right upper lung	15	13	+ 8	Negative 9 months	195	+	7	7	9
23	29	White	Moderately advanced, right lower lung	10	8	+10	Negative 6 months	62	+	8	8	1
24	33	White	Moderately advanced, right upper lung	18	15	+10	Negative 8 months	90	+	12	12	3
25	22	White	Moderately advanced, right upper lung	12	10	+14	Negative 7 months	120	+	6	6	3

E.S.R.—erythrocyte sedimentation rate. SM—streptomycin. PAS—para-aminosalicylic acid.

varying from five to twenty-five months when streptomycin was used, and from two to twenty-five months when PAS was used. In only 2 cases were these medications in use at the time of the study.

The following liver function tests were performed: cephalin flocculation, thymol turbidity, bromsulfalein (B.S.P.), total cholesterol and cholesterol esters, total protein, albumin-globulin ratio, and alkaline phosphatase.

All tests were performed with the patients in resting and fasting state. In most instances, the results represent the averages of several determinations. In performing the bromsulfalein test, 5 mg. of the dye per kg. of body weight were injected intravenously. A retention of more than 5 per cent of the dye in 45 minutes was considered abnormal. A cephalin flocculation reading of 2 plus or higher, thymol turbidity above 5 units, total serum cholesterol level above 300 mg. per 100 ml., cholesterol ester fraction below 60 per cent, total protein below 5.0 gm. per 100 ml., albumin-globulin ratio below 1.5:1, and an alkaline phosphatase value of more than 14 K.A. units were considered as indicative of impairment of liver function.

TABLE 2

ANALYSIS OF LIVER FUNCTION STUDIES IN CASES OF PNEUMOPERITONEUM

CASE NUMBER	CEPHALIN FLOCCULATION	THYMOL TURBIDITY UNITS	BROMSULFALEIN PER CENT RETENTION	PROTEINS GM/100 CC.			CHOLESTEROL MG/100 CC.			ALKALINE PHOSPHATASE K.A. UNITS
				Total	Albumin	Globulin	Total	Esters	Free	
1	Negative	6.2	3.5	—	—	—	—	—	—	—
2	Negative	7.0	5.3	6.9	4.6	2.3	199	149	50	8.7
3	Negative	1.3	7.0	6.5	4.2	2.3	257	182	75	4.7
4	Negative	7.8	2.3	7.2	4.8	2.4	292	211	81	8.5
5	Negative	3.2	4.5	6.8	4.9	1.9	284	206	78	12.1
6	Negative	3.3	1.8	6.9	4.9	2.0	257	199	58	5.4
7	Negative	2.3	6.5	6.6	4.6	2.0	280	209	71	6.4
8	Negative	3.6	1.5	6.8	4.6	2.2	297	211	86	8.2
9	Negative	6.3	4.9	6.6	4.2	2.4	291	213	78	4.7
10	Negative	4.9	4.1	7.1	4.9	2.2	460	375	85	4.4
11	3+	13.5	5.3	7.7	5.3	2.4	236	183	53	4.6
12	Negative	4.6	3.7	6.4	4.7	1.7	280	196	84	8.4
13	Negative	4.4	3.0	7.0	5.3	1.7	305	227	78	12.0
14	Negative	3.6	4.3	6.4	4.2	2.2	300	205	95	8.0
15	Negative	3.0	4.0	6.8	4.4	2.4	280	200	80	6.8
16	2+	4.8	1.5	7.3	5.0	2.3	290	210	80	8.0
17	Negative	3.0	2.0	6.8	4.8	2.0	255	190	65	5.4
18	Negative	1.5	3.7	5.9	4.0	1.9	310	230	80	5.4
19	Negative	3.4	5.9	6.8	4.3	2.5	354	230	124	8.7
20	Negative	1.3	3.0	7.2	5.0	2.2	300	230	70	10.4
21	Negative	7.0	8.0	7.0	5.0	2.0	298	210	88	10.0
22	3+	6.4	2.3	6.5	4.8	1.7	310	220	90	4.5
23	Negative	2.3	1.5	6.8	4.9	1.9	290	210	80	8.4
24	2+	5.0	4.8	7.4	5.2	2.2	254	190	64	8.0
25	Negative	2.3	4.0	7.0	5.1	1.9	320	210	110	5.4

RESULTS

In table 2 may be seen the results of the liver function tests in the 25 patients. A 2 or 3 plus cephalin flocculation was obtained in 4 cases, and a thymol tur-

bidity reading above 5 units in 7 instances. The bromsulfalein test showed a retention of 5 per cent or greater in 6 cases. The total blood protein concentration, the albumin-globulin ratio, as well as the alkaline phosphatase levels, were within the normal range in all cases. In 6 patients the total cholesterol was elevated above 300 mg. per 100 ml. The ratio of cholesterol esters to total cholesterol was within normal range in all instances.

In this series of liver function tests, consistent abnormalities were not present in any of the cases. Only one patient (Case 11) showed an abnormality in as many as three of the tests. The cephalin flocculation was 3 plus, thymol turbidity was 13.5 units, and bromsulfalein retention was 5.3 per cent. However, the values for the other tests in this case were normal.

In general the results demonstrate that long-continued pneumoperitoneum and prolonged intra-abdominal positive pressure do not cause any definite impairment in liver function. There was no significant correlation between the duration of the pneumoperitoneum, the degree of positive intra-abdominal pressure, and their effects on liver function.

DISCUSSION

Various investigators have reported a high incidence of abnormal liver function tests in cases of pulmonary tuberculosis. Levinson and Siegal (6), Kruger and Gerber (7), and Hurst, Maier, and Lough (8) found impairment in liver function, particularly in the bromsulfalein retention values, in a significant number of cases. There was a close parallel between the degree of the hepatic dysfunction, the chronicity, severity, and extent of the pulmonary disease. Patients with toxemia, emaciation, far advanced caseous-pneumonic and exudative disease showed the greatest incidence and degree of liver dysfunction.

In view of the above findings, the present study was limited to patients with well-stabilized and inactive lesions in order to exclude the adverse effect of active tuberculous disease on liver function.

Clinical observations indicate that air in the peritoneal cavity is benign in its effect on the abdominal viscera. Trimble, Eaton, and Moore (9), in a study of autopsied cases, found that long-continued pneumoperitoneum did not produce any demonstrable pathologic changes in the abdominal viscera. The only significant finding was a mild nonspecific peritoneal reaction in a few cases. Lichtman (10) stated that the pressure in the portal bed in man is approximately 10 cm. of water. This equals the average intra-abdominal pressure in the present cases of pneumoperitoneum. If the intra-abdominal air were to exert its pressure effect equally throughout the abdominal cavity, it conceivably would produce an increased resistance to portal blood flow. However, Snyder (11) emphasized that there is a reciprocal relationship between the hepatic and portal blood flow in that a compensatory increase in hepatic artery circulation occurs in the presence of a diminished portal vein flow. Bradley (12) has shown that the blood oxygen tension in the hepatic artery and portal vein is 85 and 50 per cent, respectively, and that normally the hepatic artery accounts for only 15 to 25 per cent of the total liver blood supply.

The ultimate effect of pneumoperitoneum and prolonged positive intra-abdominal pressure, therefore, may be an increased resistance to portal blood circulation, a compensatory increase in the hepatic artery blood flow, and an increase in the oxygen supply to the liver cells. This compensatory mechanism may account for the maintenance of normal liver function in therapeutic pneumoperitoneum.

SUMMARY

A study was made of liver function in 25 patients with pulmonary tuberculosis treated with pneumoperitoneum.

All patients were in good physical and nutritional status and the pulmonary disease in each case was stable and inactive. The sputum and gastric contents were negative for tubercle bacilli for periods varying from six to fourteen months at the time the liver function studies were conducted.

All variables having the potentiality of causing liver function impairment were eliminated as far as possible. Thus, the present study was concerned with the effect of pneumoperitoneum and elevated intra-abdominal pressure, per se, upon liver function.

The duration of the pneumoperitoneum varied from six to twenty-one months, the average intra-abdominal pressure was plus 12 cm. of water throughout the maintenance of this therapy.

The results of the present study indicate, therefore, that long-continued pneumoperitoneum and prolonged increased intra-abdominal pressure did not produce impairment of liver function.

SUMARIO

El Efecto del Neumoperitoneo Terapéutico en la Tuberculosis Pulmonar sobre la Función Hepática

En 25 tuberculosos pulmonares tratados con el neumoperitoneo se ejecutó un estudio de la función hepática.

Todos los sujetos se hallaban en buen estado físico y nutritivo y en todos la afección pulmonar estaba estabilizada e inactiva. El esputo y el contenido gástrico habían sido negativos para bacilos tuberculosos durante períodos de tiempo que variaban de seis a catorce meses en la fecha en que se hicieron los estudios de la función hepática.

En todo lo posible, se eliminaron todas las variables potencialmente capaces de ocasionar disfunción hepática. El estudio actual versó, pues, sobre el efecto del neumoperitoneo y de la hipertensión intraabdominal, en sí mismos, sobre la función del hígado.

La duración del neumoperitoneo varió de seis a veintiún meses y la presión intraabdominal media fué de +12 cm. de agua durante toda la aplicación de esta terapéutica.

Los resultados del estudio actual indican, por consiguiente, que un neumoperitoneo largo y una prolongada hipertensión intraabdominal no afectaron adversamente la función hepática.

RESUME

Action de la pneumopéritonéothérapie pour tuberculose pulmonaire sur la fonction hépatique

Une étude de la fonction hépatique a été effectuée chez 25 malades atteints de tuberculose pulmonaire et traités par le pneumopéritoine.

Chez chacun de ces malades, l'état général et la nutrition étaient satisfaisants et l'affection pulmonaire était stabilisée. Au cours des recherches sur la fonction hépatique, les

crachats et le liquide gastrique furent négatifs pendant des périodes variant de six à quatorze mois.

Toutes les variables susceptibles de créer un potentiel de dysfonction hépatique ont été éliminées dans la mesure du possible. Cette étude s'est donc attachée à déterminer les effets du pneumopéritoine et de l'augmentation de la pression intra-abdominale, en soi, sur la fonction hépatique.

La durée du pneumopéritoine variait de six à vingt-et-un mois, la valeur moyenne de la pression intra-abdominale atteignait +12 cm. d'eau pendant la période où cette thérapie était poursuivie.

En conséquence, les résultats de l'étude actuelle indiquent que le maintien prolongé d'un pneumopéritoine avec accroissement de la pression intra-abdominale n'entrainait aucune altération de la fonction hépatique.

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THE INFLUENCE OF CORTISONE AND STREPTOMYCIN ON EXPERIMENTAL TUBERCULOSIS IN THE ALBINO RAT[1, 2, 3]

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INTRODUCTION

Recent reports indicate that cortisone and corticotropin (ACTH) alter the natural course of experimental tuberculosis in mice, rats, guinea pigs, and rabbits (1–4). These compounds have been shown to favor the progression of the disease and in some instances have altered the histologic manifestations. In addition, it has been reported that cortisone diminished the therapeutic effect of streptomycin administered to tuberculous guinea pigs (3, 5). Because these studies in guinea pigs were carried out in a highly susceptible host, it was considered to be of interest to learn whether cortisone would similarly modify the therapeutic effectiveness of streptomycin on tuberculosis in the albino rat, which is resistant to tuberculous infection.

MATERIALS AND METHODS

Forty albino rats, each weighing 150 gm., were divided into four groups; one group was injected intraperitoneally with 1.0 mg. of H37Rv (virulent human tubercle bacilli); another group was likewise injected with 1.0 mg. of H37Rv, and also received 5 mg. of cortisone acetate (Merck) intramuscularly daily for the duration (sixty days) of the experiment. A third group, injected with 1.0 mg. of H37Rv intraperitoneally, received 5 mg. of cortisone and 10 mg. of streptomycin chloride complex intramuscularly daily for the duration of the experiment. A fourth group, similarly infected with H37Rv, was treated with 10 mg. of streptomycin daily.

RESULTS

Eight of 10 infected animals which received cortisone alone died within sixty days. At the end of this time, all of the infected animals which did not receive cortisone and also those which received cortisone plus streptomycin were living and well (table 1). The infected rats which received cortisone showed an average weight loss of 50 gm., whereas the animals which were treated with streptomycin had an average weight gain of 75 gm. The histologic findings in this experiment are presented below.

[1] From the Tuberculosis Research Laboratory, Veterans Administration Hospital, and the Departments of Medicine and Pathology, Emory University School of Medicine, Atlanta, Georgia.

[2] Presented, in part, before the Medical Session, as part of the symposium on *Cortisone and ACTH*, at the 47th annual meeting of the National Tuberculosis Association, Cincinnati, Ohio, May 15, 1951.

[3] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

Rats infected with tubercle bacilli: In the lungs of the rats which had been inoculated with tubercle bacilli there were some small, mostly subpleural, foci of granulomatous pneumonia, a few of which secondarily involved the bronchioles (figure 1, left). The inflammatory process consisted chiefly of large mononuclear cells of the epithelioid-cell type, large vacuolated macrophages, and a few

TABLE 1

EFFECT OF CORTISONE AND STREPTOMYCIN ON SURVIVAL TIME OF RATS INFECTED WITH HUMAN TUBERCLE BACILLI

NUMBER OF ANIMALS	GROUP	DAY OF DEATH	NUMBER OF SURVIVORS AT 60 DAYS
10	Tubercle bacilli	17, 17, 18, 33, 40, 46, 48, 50	10
10	Tubercle bacilli—cortisone		2
10	Tubercle bacilli—streptomycin		10
10	Tubercle bacilli—streptomycin —cortisone		10

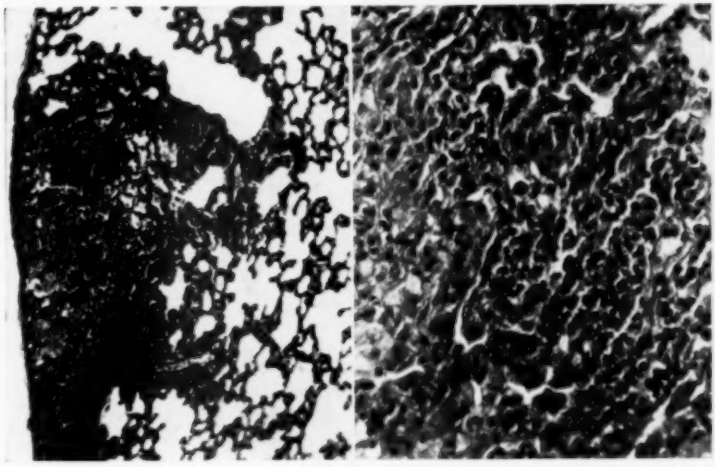

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FIG. 1. Rat infected with tuberculosis. (Left) Illustrating small subpleural focus composed of large mononuclear cells and lymphocytes. ×75. (Right) Illustrating the cells of the infiltrate at higher magnification. ×330. Hematoxylin—eosin.

multinucleated cells of the foreign-body giant-cell type. Lymphocytes were encountered in fair numbers and the process had elicited some fibroblastic response (figure 1, right). There was no caseation but acid-fast bacilli were easily found. Peripheral to the granulomatous foci, the lungs showed some interstitial pneumonitis with septa infiltrated by large mononuclear cells of the same type as described above.

Rare minute granulomata consisting of a few large mononuclear cells were seen in the liver where they elicited minimal or no fibroblastic response. The lesions were found chiefly in portal and periportal areas. They contained rare acid-fast bacilli, but there was no necrosis.

A few minute granulomata similar to those in the liver were seen scattered throughout the spleen. In these many acid-fast bacilli were found, but no caseation was noted.

Rats infected with tubercle bacilli and treated with cortisone: The lungs of rats which had received tubercle bacilli and had been maintained on cortisone displayed widespread, diffuse, granulomatous pneumonia as well as interstitial

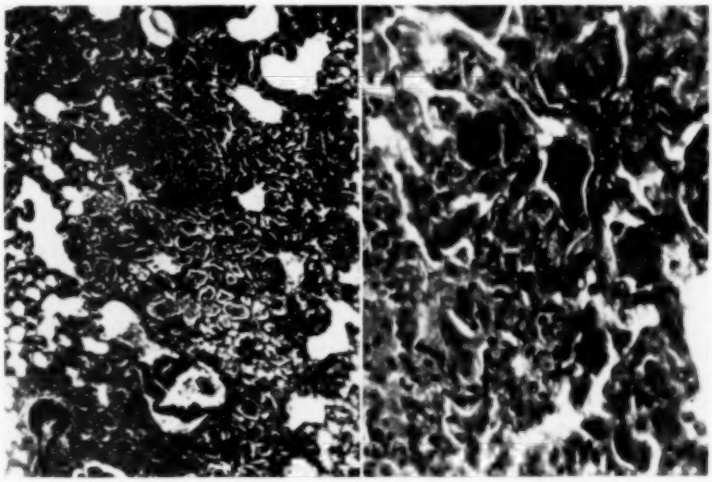

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FIG. 2. Rat treated with cortisone and infected with tuberculosis. (Left) Showing a spreading lesion involving a bronchus (bottom center). ×75. (Right) Revealing large mononuclear cells and frequent multinucleated cells, some of the Langhans' type. Only few lymphocytes are present. ×330. Hematoxylin—eosin.

pneumonitis (figure 2, left). The inflammatory cell infiltrate consisted chiefly of large mononuclear cells with foamy cytoplasm, but many so-called epithelioid cells were noted. Some multinucleated cells of the Langhans' type were present in areas of epithelioid cell pneumonia. In the granulomatous lesions, comparatively small numbers of lymphocytes and practically no fibroblastic proliferation were present (figure 2, right). Acid-fast bacilli were extremely numerous. Secondary involvement of the bronchi was common and had led to definite bronchiogenic dissemination. In the 8 animals which died before the sixtieth day of the experiment, an extensive acute purulent pneumonia with large masses of gram-positive bacteria complicated the picture (figure 3). These organisms did not take the Ziehl-Neelsen stain and probably represented a mixed flora with coccal

and bacillary forms. In addition, tuberculous lesions similar to those described above were present and contained typical acid-fast bacilli. In the spleen there were marked lymphoid depletion and many granulomata composed predominantly of epithelioid cells. These lesions lacked peripheral cellular response. Similar granulomata were encountered in the liver where they were, however, much less numerous than in the spleen. The splenic and hepatic lesions contained numerous tubercle bacilli but were without caseation. The 2 animals which were sacrificed on the sixtieth day showed similar, although somewhat less extensive, tuberculous lesions containing innumerable acid-fast bacilli.

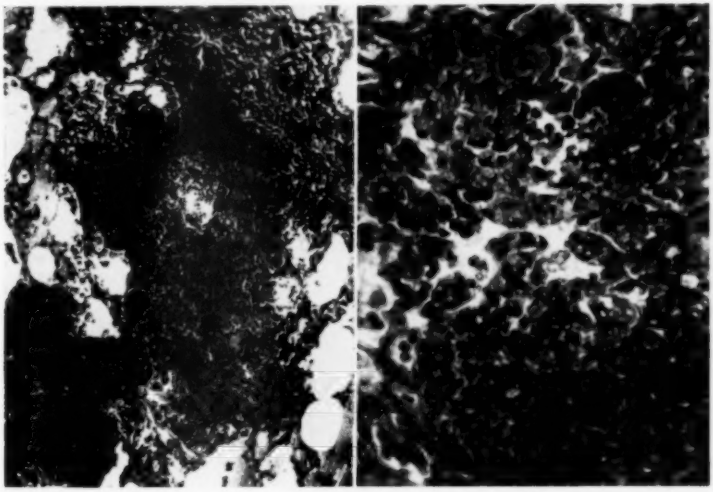

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FIG. 3. Rat treated with cortisone and infected with tuberculosis. A marked secondary pyogenic infection has occurred. (Left) Showing an area of bacterial bronchopneumonia with abscess formation obscuring a tuberculous lesion. ×75. (Right) Revealing polymorphonuclear leukocytes of the secondary infection as well as an infiltrate of large mononuclear cells representing the tuberculous lesion. ×330. Hematoxylin—eosin.

Rats infected with tubercle bacilli and treated with cortisone and streptomycin: The pulmonary lesions of rats inoculated with tubercle bacilli and treated with both cortisone and streptomycin did not differ appreciably from those observed in tuberculous animals treated with cortisone alone (figure 4, left). The lymphocytes in the inflammatory cell infiltrate were possibly more numerous but, if a difference existed, it was slight (figure 4, right). Many acid-fast bacilli were present in the lesions. Secondary bacterial pneumonia was not encountered.

The spleen was the site of lymphoid depletion and numerous tubercles with many bacilli. Some tubercles containing many acid-fast bacilli were also found in the liver.

Rats infected with tubercle bacilli and treated with streptomycin: All of the ani-

mals which had been infected with tubercle bacilli and treated with streptomycin survived the sixty-day period of the experiment, gained weight, and manifested no evidence of illness. There were no gross lesions at autopsy. Only a few animals were studied histologically. In the lungs rare granulomas composed predominantly of foamy macrophages were found. In the spleen rare small granulomas composed of epithelioid cells were seen. No lesions were seen in the liver. The lesions contained rare acid-fast organisms.

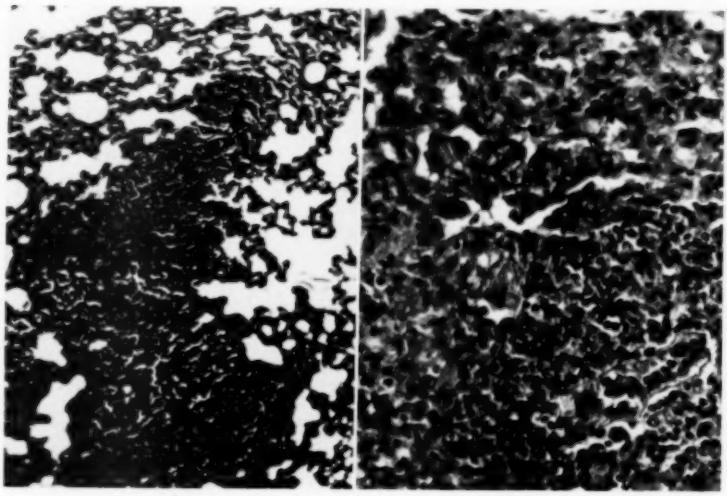

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FIG. 4. Rat treated with cortisone and streptomycin and infected with tuberculosis. (Left) Showing a large tuberculous lesion extending into a small bronchus (top right). ×75. (Right) Revealing chiefly large mononuclear cells and only few and scattered lymphocytes. ×330. Hematoxylin—eosin.

DISCUSSION

It is interesting that streptomycin protected the albino rat from the deleterious effect of cortisone in so far as survival from infection is concerned. In this respect the findings differ from the results obtained with guinea pigs. The extent of the tuberculosis was greater in the susceptible host receiving cortisone even when adequate doses of streptomycin were administered (3, 5). The histologic study in the albino rat revealed that streptomycin when administered with cortisone did not completely prevent the formation of tubercles in the lungs, liver, and spleen, even when treatment with the drug was begun on the day of infection and continued for the duration of the study (sixty days). However, the infection was less extensive in streptomycin-treated than in untreated animals. These findings differ from observations in this laboratory and by others when guinea pigs are treated in a similar manner.

It has been shown that a form of pseudotuberculosis produced by *C. pseudotuberculosis murium* may emerge in rats which are maintained on cortisone (6).

Cultures made of organs of the strain of rats in the present experiment (Sherman albino, Rockland Farms, New York) did not reveal *Corynebacteria*, but it is probable that streptomycin may have influenced the survival time of the rats receiving cortisone by eliminating streptomycin-sensitive bacteria other than tubercle bacilli.

In previous similar experiments (2), 2 of 10 albino rats which received cortisone alone for fifty-two days had evidence of pyogenic lung infection. It has been demonstrated that cortisone renders animals more susceptible to spontaneous and experimentally induced pyogenic infection (7). It is interesting that, of the 10 rats in the experiment now reported infected with tubercle bacilli and treated with cortisone, 8 developed severe pyogenic pulmonary infection. It seems possible that the pulmonary tuberculous lesions increase the susceptibility of these animals to secondary pyogenic lung infection. Although this secondary infection may have been responsible for death, there is still histologic evidence of more extensive tuberculosis in this group than in the group of rats infected with tubercle bacilli and not treated with cortisone.

This experiment suggests that host resistance remains one of the principal factors in the pathogenesis of tuberculosis. Results obtained in one species of animal do not necessarily apply to others. One may postulate that human beings who vary in their native resistance to tuberculosis may in some instances respond to adrenocorticoid therapy either like the guinea pig or like the rat, depending on their individual susceptibility to tubercle bacilli.

SUMMARY

Albino rats infected with human-type tubercle bacilli and treated with cortisone develop more severe tuberculosis than infected animals not receiving this hormone.

Albino rats infected with tubercle bacilli and treated with cortisone and streptomycin have infection comparable to untreated animals infected with tubercle bacilli.

Therapeutic doses of streptomycin diminish the infection in the albino rat infected with tubercle bacilli. Streptomycin does not, however, prevent the formation of minimal lesions in the lungs, liver, and spleen.

SUMARIO

El Influjo de la Cortisona y la Estreptomicina sobre la Tuberculosis Experimental en la Rata Albina

Las ratas albinas infectadas con bacilos tuberculosos y tratadas con cortisona manifiestan tuberculosis evolutiva que culmina en la muerte de las mismas.

Dosis terapéuticas de estreptomicina protegen a la rata albina infectada con bacilos tuberculosos contra la muerte debida a tuberculosis. Sin embargo, dicho antibiótico no impide completamente la formación de lesiones en los pulmones, hígado y bazo.

RESUME

L'influence de la cortisone et la streptomycine sur la tuberculose expérimentale du rat albinos

Lorsque le rat albinos inoculé avec le bacille tuberculeux est traité par la cortisone il en résulte une tuberculose évolutive, qui cause la mort de l'animal.

Des doses thérapeutiques de streptomycine protègent contre la mort par tuberculose le rat albinos inoculé avec le bacille tuberculeux. Toutefois, cet antibiotique ne prévient pas complètement la formation de lésions dans le poumon le foie et la rate.

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COMPARISON OF EXPERIMENTAL TUBERCULOSIS IN CORTISONE-TREATED AND ALLOXAN-DIABETIC ALBINO RATS[1, 2]

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INTRODUCTION

A generally accepted clinical impression is that diabetes lowers the resistance of a patient to tuberculosis and to other infections. Despite this observation, very little experimental work has been done on the influence of diabetes upon the progress of tuberculosis. Steinbach and Duca (1), using rats rendered hyperglycemic by pancreatectomy, have reported an increased susceptibility to tuberculous infection which appeared to vary directly with the degree of hyperglycemia. In addition, these investigators noted that the subcutaneous injection of 20 per cent glucose twice daily also appeared to depress the resistance of the rat to tuberculosis, although they were careful to state that the induced hyperglycemia may not have been the sole cause of this change in resistance. Some evidence has been obtained which tends to elucidate the mechanism by which diabetes causes a reduction in specific defense reactions of the body. DaCosta and Beardsley (2) have found a diminution in the ability of leukocytes of diabetics to phagocytize tubercle bacilli, *Streptococci*, and *Staphylococci*. Richardson (3) has reported that poorly controlled diabetics are less able to form agglutinins after injections of typhoid vaccine than are normal individuals or well-controlled diabetics. Moen and Reimann (4) and Bates and Weiss (5) have also presented evidence of a diminution of antibody formation in the diabetic.

In relation to tuberculosis, Long (6) has suggested that the susceptibility of the diabetic may be associated with the increased breakdown of fat in the diabetic accompanied by liberation of glycerol. Long and Vorwald (7) presented some evidence that glycerol tends to increase the susceptibility of the rat to tuberculosis.

Cortisone administration, like diabetes, appears to lower the natural resistance to infectious agents. Clinical observations have revealed that the administration of cortisone or corticotropin (ACTH) to patients with pulmonary tuberculosis may result in the development of miliary lesions (8). Experimentally, Germuth and Ottinger (9) have reported that cortisone markedly diminished antibody formation in the rabbit. Conversely, Mirick (10) found that antibody formation to pneumococcal polysaccharides was produced just as promptly and in as high a titer in patients treated with cortisone as in untreated controls. Histologic

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[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

studies have demonstrated a pronounced depression of the inflammatory response following the administration of this hormone (11, 12). Shwartzman (13) has described an enhancement of poliomyelitis in experimental animals given cortisone, while Kligman and associates (14) have reported a variety of experimental infections which are adversely affected by cortisone administration.

In a recent study, Michael, Cummings, and Bloom (15) have produced overwhelming tuberculosis in the albino rat, normally a highly resistant animal, through the use of daily injections of cortisone for a prolonged period. Other investigators (16, 17) have found the same enhancement of the progress of tuberculosis in cortisone-treated mice and guinea pigs. However, Lurie and co-workers (18), working with tuberculosis in the rabbit, described greater localization of tubercle bacilli in the lungs following cortisone administration, despite the fact that their cortisone-treated animals had greater numbers of tubercle bacilli in the lesions than the controls. In addition to their similarities relating to a reduction in natural resistance, diabetes and cortisone administration also have in common a disturbance in carbohydrate metabolism. Using similar doses of cortisone, as did Michael and associates, Ingle and his co-workers (19) have produced marked glycosuria in rats fed high carbohydrate diets by stomach tube. As no metabolic studies were done by Michael in his experiments on tuberculosis in the rat, although they were on normal diets, the possibility that his animals had been made clinically diabetic must be considered. The present study was designed to repeat the work done on the production of tuberculosis in the cortisone-treated rat with measurements of the degree of glycosuria, to study the course of experimental tuberculosis in the alloxan-diabetic rat, and to contrast the disease produced in the two groups.

MATERIALS AND METHODS

Forty male albino rats (Sherman), whose average weight was 200 gm., were used in this experiment. Twenty of these rats were given intravenous injections of 40 mg. of alloxan per kg. of body weight. Eight of the 20 alloxanized rats died within ten days after alloxan administration due to alloxan toxicity, i. e., renal damage, hypoglycemia, et cetera. Preliminary screening for glycosuria was done by means of "Clinitest"[1] tablets. Animals having glycosuria were placed in metabolism cages, and a twenty-four-hour urine specimen was collected and the glucose content and urine volume recorded. The criteria used to determine the presence of diabetes were glycosuria and polyuria. Ten days after alloxan administration, the remaining 14 diabetic rats and 20 normal nondiabetic rats were injected intraperitoneally with 4 mg. of human-type tubercle bacilli (H37Rv). During the first week of infection 5 more of the alloxanized rats died, presumably due to alloxan toxicity. The remaining 9 rats of this group survived, although 2 of these 9 animals were found not to have sustained glycosuria or polyuria and were eliminated from the study. At the termination of the experiment these 2 animals did not differ in the gross or microscopic pathology from that previously found in untreated tuberculous rats.

The above-mentioned 20 nondiabetic rats were divided into two groups. Ten rats were given 5 mg. of cortisone acetate (Merck) daily by the intramuscular route, and the other 10 animals received 1 mg. of cortisone acetate daily during the course of the experiment. Before these animals were infected or treated with cortisone, they were also placed in

[1] Proprietary name for $CuSO_4$, NaOH, Na_2CO_3, citric acid, Ames Co. Inc., Elkhart, Indiana.

TABLE 1

SURVIVAL TIME FOR ALL GROUPS OF ANIMALS

NUMBER OF ANIMALS	GROUP	DAY OF DEATH	NUMBER OF SURVIVORS AT 60 DAYS
7	Tubercle bacilli plus alloxan	22, 24, 26, 29, 37	2
5	Alloxan only		5
10	Tubercle bacilli plus 5 mg. of cortisone	30, 37, 40, 41, 45, 51, 52	3
10	Tubercle bacilli plus 1 mg. of cortisone		10
10*	Tubercle bacilli only		10
10*	5 mg. of cortisone only		10

* Data from other experiments (15, 21).

TABLE 2

RELATIVE NUMBER OF ACID-FAST BACILLI ENCOUNTERED IN THE VARIOUS ORGANS OF THE TREATED AND UNTREATED ANIMALS

GROUP	LUNG	LIVER	SPLEEN
5 mg. of cortisone plus tubercle bacilli	++++	+++	+++
1 mg. of cortisone plus tubercle bacilli	+++	+	+
Alloxanized plus tubercle bacilli	++	+	+
Tubercle bacilli only	+	±	±
Alloxan only	0	0	0
5 mg. of cortisone only	0	0	0

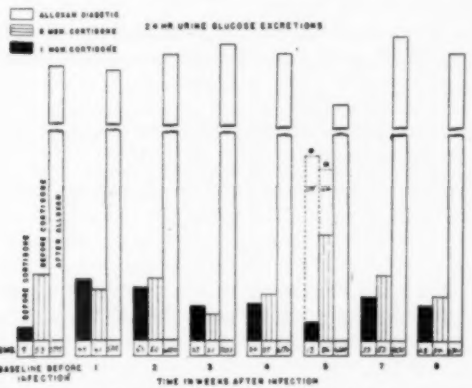

FIG. 1. Quantitative determinations of glucose excreted during twenty-four hours expressed in milligrams per 100 milliliters.

* Dotted lines in fifth-week data represent the increased amount of glucose excreted after forced feeding with 20 per cent sucrose.

metabolism cages, and glucose concentration and urine volumes were recorded to establish a base line.

All animals were fed on a regular stock diet, consisting of Rockland mouse diet and water *ad libitum*. At weekly intervals during the experiment, each group was weighed and the urine volume and glucose excretion recorded (tables 1 and 2). Glucose concentrations were established by the anthrone method performed on Folin filtrates of the urine (20). During the fifth week of the experiment, the two cortisone groups were fed on a special diet of stock food plus 20 per cent sucrose solution in place of the regular water supply. This forced feeding of a high carbohydrate diet elevated the degree of glycosuria in the cortisone-treated groups; the amount of this elevation is indicated by the dotted lines in figure 1.

Data used for comparison with the results of the present experiment were taken from other experiments in this laboratory which utilized the same strain of rat and the same infecting organism (H37Rv). In these experiments, 5 mg. of cortisone daily were given to normal rats with no apparent ill effects except a slight loss of weight during the sixty-day

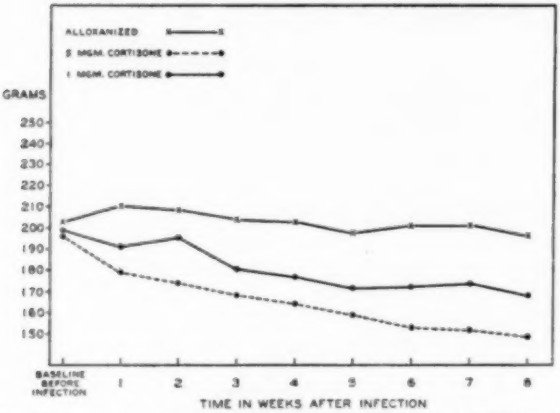

FIG. 2. Weight curves of rats infected with tubercle bacilli and treated with cortisone or alloxan.

period. The complete data on these previous experiments are not included in the present paper since they have been reported previously (15, 21). Likewise, 5 alloxanized uninfected rats served as controls for survival time studies. Blood glucose determinations (Nelson's method) at the end of sixty days indicated that the hyperglycemia was sustained (mean value 231.1 mg. per 100 cc.).

RESULTS

The survival time for all groups of animals is indicated in table 1. Previous experiments in this laboratory have shown that 100 per cent of the control group (normal rats), infected with tubercle bacilli only, survived. Thirty per cent of the group infected with tubercle bacilli and treated daily with 5 mg. of cortisone acetate survived; whereas, 100 per cent of those animals infected and treated with 1 mg. of cortisone survived. In the present experiment, 30 per cent of the rats infected with tubercle bacilli and rendered diabetic by alloxan survived until the termination of the experiment at sixty days. All of the uninfected

alloxanized animals survived sixty days. It may be noted from figure 2 that both the 1 mg. and the 5 mg. cortisone-treated animals lost weight, whereas the alloxan-diabetic animals did not lose weight. There were only slight differences noted in the gross pathology between the different groups of treated animals.

Gross Pathology

Rats infected with tubercle bacilli and treated with 5 mg. of cortisone daily: The lungs of the animals infected with tubercle bacilli and treated with cortisone were studded with large caseous masses involving the whole lung and, in many cases, the thoracic wall and the diaphragm. There were many fairly large nodules found on the liver of the animals of this group. The spleen was usually enlarged from two to three times normal size and was studded with small gray lesions. The omentum was considerably involved and usually adhered to the spleen and peritoneal wall.

Rats infected with tubercle bacilli and treated with 1 mg. of cortisone daily: With the exception of 2 animals having only slight lung involvement, the gross pathology in the group of infected rats which received 1 mg. of cortisone was quite comparable to the above group of rats which received 5 mg. of cortisone.

Rats infected with tubercle bacilli and rendered diabetic by alloxan: The lungs of animals infected with tubercle bacilli and rendered diabetic by alloxan revealed many nodules and lesions extending to the chest wall and diaphragm. There was less caseous material in the lungs of this group than in the lungs of the above-mentioned groups. The liver, spleen, and omentum were involved in much the same manner and to the same extent as the cortisone-treated groups.

Microscopic Pathology

Rats infected with tubercle bacilli: The lungs of the animals which had been infected with tubercle bacilli and had received no cortisone or alloxan contained clusters of mononuclear cells, surrounded in some areas by lymphocytes and with little or no alveolar exudate or surrounding edema. The over-all picture was that of an orderly granulomatous process. Acid-fast stains demonstrated a few organisms located chiefly within the mononuclear cells comprising the tubercles. The spleens contained small collections of epithelioid cells in the center of the Malpighian corpuscles. Acid-fast bacilli were sparse and were located principally within these cells. No lesions were noted in the liver and only small numbers of acid-fast organisms were found in the Kupffer cells. These microscopic sections were taken from previous experiments (15, 21).

Rats infected with tubercle bacilli and treated with cortisone: The lungs of the group of animals infected with tubercle bacilli and treated with cortisone contained many lesions which varied markedly in size, some involving only one or two contiguous alveoli, but others occupying an area represented by a low-power microscopic field. In some of the latter lesions there were small focal areas of caseation necrosis and extensive necrosis of the mononuclear cell infiltrate in the alveoli. The remainder of the lesion is characterized by a heavy infiltrate of the alveoli lumen and interstitial tissue with macrophages. Many of the larger

lesions were characterized by an extensive infiltration of the lung parenchyma by macrophages, many of which were epithelioid in configuration. A few lymphocytes were seen in these lesions. The lymphoid tissue of these lungs was prominent and a few plasma cells were seen in the aggregate of lymphocytes. Smaller lesions were composed of aggregates of macrophages and lymphocytes. Rare multinucleated macrophages similar to Langhans' giant cells were seen. Acid-fast stains revealed numerous bacilli throughout the lung which appeared in the exudate and in interstitial areas. The organisms were much more plentiful than in the groups not receiving cortisone. The spleens showed numerous small lesions composed almost exclusively of epithelioid cells. Little, if any, necrosis was noted. Numerous acid-fast bacilli were found in the supporting stroma. In most of the liver sections no lesions were identified. Rarely a few granulomatous lesions similar to those seen in the spleen were seen. The liver contained acid-fast bacilli lying principally within the Kupffer cells. There were several areas of polymorphonuclear infiltration in the lungs suggesting secondary infection.

Rats infected with tubercle bacilli and rendered diabetic with alloxan: The microscopic sections of the organs from animals which had been infected with tubercle bacilli and rendered diabetic with alloxan revealed no significant basic differences in terms of histologic characteristics from the lesions of the cortisone-treated groups of animals. However, there were definitely fewer lesions in the alloxanized group and considerably fewer acid-fast bacilli than in the cortisone-treated group. The amount of acid-fast bacilli found in the different organs of each group may be seen in table 2. Cultures of the spleen, liver, and lungs were made on several occasions, employing Löwenstein-Jensen, blood agar, and Löffler's serum slants. *M. tuberculosis* was recovered from all of the organs. Attempts to isolate *C. pseudotuberculosis murium* or *P. pseudotuberculosis* were unsuccessful.

DISCUSSION

The susceptibility of the albino rat to tuberculosis appears to be enhanced by the administration of cortisone and similarly by rendering them diabetic through the use of alloxan. Although the enhancement of tuberculosis in rats rendered diabetic by pancreatectomy has previously been demonstrated, diabetes produced by alloxan should obviate the objections raised by Rich (22), to the earlier work, that pancreatectomy removes other pancreatic enzymes than insulin.

Although none of the 1 mg. cortisone-treated group died, at the termination of the experiment, grossly and microscopically, the disease was almost as extensive as in the 5 mg. cortisone group. This suggests that, although 1 mg. of cortisone daily causes sufficient depression of resistance to permit progress of infection, the death of the animals in the 5 mg. cortisone-treated group may be due to the infection plus the more extensive metabolic alterations caused by the higher dosage. Likewise, the high mortality rate in the alloxanized animals with less extensive disease than was found in the 1 mg. or 5 mg. cortisone-treated groups would seem to indicate some extensive alterations in the metabolism as well as

a lowering of the natural host resistance by suppression of the acute inflammatory reaction.

SUMMARY

Rats injected with virulent tubercle bacilli did not die of tuberculosis or develop any progressive infection. A majority of the rats similarly infected but treated with cortisone or rendered diabetic by alloxan either died or developed a progressive tuberculous infection.

No correlation was established between the amount of glycosuria and degree of disease.

There were marked histologic differences between rats infected with tubercle bacilli and untreated and those treated with cortisone or alloxanized. Little difference was noted, however, in the histologic pattern of infection between the the cortisone- and alloxan-treated groups.

Considerably more acid-fast organisms were seen in the organs of rats treated with cortisone than in the control or alloxan-treated groups.

The influence of cortisone and diabetes on experimental tuberculosis appears to be a lowering of the natural host resistance.

SUMARIO

Comparación de la Tuberculosis Experimental en las Ratas Albinas Tratadas con Cortisona y las Diabetizadas con Aloxán

Ratas inyectadas con bacilos tuberculosos virulentos no murieron de tuberculosis ni manifestaron infección evolutiva. La mayoría de las infectadas en forma semejante, pero tratadas con cortisona o diabetizadas con aloxán, o bien murieron o manifestaron infección tuberculosa evolutiva.

No se estableció la menor correlación entre la intensidad de la glucosuria y la gravedad de la enfermedad.

Hubo decididas diferencias histológicas entre las ratas infectadas con bacilos tuberculosos y no tratadas y las tratadas con cortisona o aloxanizadas. No obstante, notóse poca diferencia en el patrón histológico de la infección entre las ratas tratadas con cortisona y con aloxán.

Observáronse muchos más microbios ácidorresistentes en los órganos de las ratas tratadas con cortisona que en los grupos testigos o tratados con aloxán.

El influjo de la cortisona y de la diabetes sobre la tuberculosis experimental parece consistir en disminución de la resistencia natural del huésped.

RESUME

Etude comparative dans la tuberculose expérimentale de rats albinos traités par la cortisone ou rendus diabétiques par l'allozane

Des rats inoculés avec des bacilles tuberculeux virulents ne sont pas morts de tuberculose en n'ont pas présenté la forme évolutive de l'infection. La majeure partie des rats inoculés de la même manière, mais traités par la cortisone ou rendus diabétiques par administration d'alloxane, a succombé ou a présenté une forme évolutive de l'infection tuberculeuse.

Il n'a pas été établi de corrélation entre la valeur de la glycosurie et le degré de l'infection.

Des différences histologiques marquées existaient entre les rats inoculés avec le B.K. et non traités et ceux traités par la cortisone ou soumis à l'alloxane. Toutefois, il y avait pue

de différences histologiques dans les aspects de l'infection entre le groupe traité par la cortisone et celui traité par l'alloxane.

Le nombre de bacilles acido-résistants observés dans les organes des rats traités par la cortisone était considérablement plus élevé que chez les animaux-témoins ou chez ceux traités par l'alloxane.

Il apparaît que l'influence de la cortisone et du diabète sur la tuberculose expérimentale détermine une réduction de la résistance naturelle de l'hôte.

Acknowledgment

We are indebted to Dr. C. Merrill Whorton, Chief, Laboratory Services, Veterans Administration Hospital, Chamblee, Georgia, for his review and assistance in the interpretation of the histologic findings in this experiment. We are also indebted to Misses Mary Schumpert and Ruth Limbert for their technical assistance.

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DIHYDROSTREPTOMYCIN SULFATE IN PULMONARY TUBERCULOSIS[1,2]

Report on Neurotoxicity

STANTON TICE ALLISON

(Received for publication September 14, 1951)

INTRODUCTION

In 1949 Volk, Vitagliano, and the writer (1) reported observations in the treatment of 20 patients with pulmonary tuberculosis who had received 2 to 3 gm. of dihydrostreptomycin daily for 90 days. Compared with streptomycin, this new hydrogenated product (dihydrostreptomycin) appeared much less toxic in every respect save one, namely, its toxicity for the auditory apparatus. There were no skin reactions, no high eosinophilias, no blood dyscrasias, and only one case of loss of vestibular function. In the period following cessation of the drug, from one week to two months post treatment, however, 4 patients (20 per cent) developed definite audiometric loss and tinnitus; clinical deafness was apparent in 3. In this early study, dihydrostreptomycin hydrochloride was used for the first two months and dihydrostreptomycin sulfate for the third month.

Previous to the publication of the above paper, one of the writers (R. V.) had presented a preliminary paper on this study at the Seventh Streptomycin Conference of the Veterans Administration, held in Denver in April 1949. These findings of deafness in a percentage of those treated were confirmed by Romansky and his associates (2), who likewise presented a paper on the same subject at this conference. These investigators observed 22.9 per cent deafness in their series of 61 cases in which the hydrochloride salt of dihydrostreptomycin was used. A later follow-up report by these authors (3) revealed deafness in 40 per cent of 82 cases studied. Of 33 deafened patients, 9 received dihydrostreptomycin sulfate only; the remainder received the hydrochloride salt.

In 1949 Carr and his associates (4) presented a paper at the Atlantic City conference of the American College of Chest Physicians in which they reported their observations of 35 patients treated with dihydrostreptomycin hydrochloride for periods of 10 to 150 days. There was slight impairment of hearing in only 2 cases. This paper was discussed by the present writer (5) who noted that one patient had been treated for 150 days, one patient for 120 days, one patient for 64 days, while all the remainder (32 patients) had been treated for 60 days or less. Hence, it appeared that it was the time element which accounted for the discrepancy in the auditory toxicity observed in these two studies: the one at the Mayo Clinic; the other at the Veterans Administration Hospital, Rutland Heights.

Since these reports appeared there has been a great deal of controversy on the

[1] From the Tuberculosis Service, Veterans Administration Hospital, Rutland Heights, Massachusetts.

[2] Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the author.

subject of incidence of toxicity for the auditory apparatus produced by dihydrostreptomycin. Lincoln and his associates (6), in December 1950, reported the treatment of 64 patients with dihydrostreptomycin for 56 to 90 days in a dosage of 40 mg. per kg. of body weight. In some cases the hydrochloride salt was used, and in other cases the sulfate. They found no demonstrable loss in acuity with-

TABLE 1

AUDITORY STATUS OF 20 PATIENTS GIVEN DIHYDROSTREPTOMYCIN SULFATE 1 GRAM DAILY FOR FOUR MONTHS

PATIENT	AUDITORY ACUITY		LATE FOLLOW-UP		REMARKS
	End of Treatment	Four or Five Months Post Treatment	Months Post Treatment	Acuity	
L. C.	A, C	A, C			
P. R.	A, C	A, C	6	A, C	
T. S.	A, C	A, C	12	A, C	
P. E.	A, C	A, C			
D. B.	A, C	C			Left against medical advice four months post treatment
J. H.	A, C	C			Died four months post treatment
M. Q.	A, C	A, C	7	A, C	
F. T.	A, C	A, C			
F. M.	A, C	A, C	6	C	
J. A.	A, C	A, C			
R. P.	A, C	A, C			
P. G.	A, C	A, C	12	A, C	
J. K.	A, C	A, C	12	A, C	
L. B.	A, C	A, C	9	A, C	
G. L.	A, C	C	9	A, C	
E. D.	C				Died at end of treatment
J. M.	A, C	A, C			
S. U.	This patient was 75 per cent deaf before treatment and showed no further audiometric loss				
J. D.	A, C	A, C			
W. R.	A, C	A, C			

A = No loss audimetrically of 30 decibels or more at frequencies of 128, 256, 512, 1,042, 2,048, and 4,096 per second.

C = No hearing loss clinically as tested by spoken voice, whispered voice, watch tick.

in the normal hearing range three to ten months after cessation of the drug therapy.

In March 1951 O'Connor and his associates (7) reported the treatment of 11 patients with dihydrostreptomycin for three months and 21 patients for six months. Three patients in the first group, who had received the hydrochloride alone, became deaf. In the second group of patients, 14 of 21 became deaf and 2 of these, who had received the hydrochloride alone for six months, became "stone" deaf.

Hawkins (8) reported an interesting study on the neurotoxic action of streptomycin and of the two salts of dihydrostreptomycin on the auditory system of cats. He concluded that the neurotoxic action of streptomycin on the auditory system of the cat is less constant than on the vestibular system and that it occurs only when large doses of streptomycin are used. This has been borne out in humans. Hawkins likewise found that dihydrostreptomycin sulfate had no neurotoxic effect upon the auditory system of the cat, whereas dihydrostreptomycin hydrochloride in equal doses caused slight but definite damage. He attributed the differences in neurotoxicity between the two salts of dihydrostreptomycin to impurities in the hydrochloride rather than to inherent differences between the two salts.

PLAN OF INVESTIGATION

For some time it had been apparent to members of the medical staff at the Rutland Heights Veterans Hospital that some patients in the older age group would develop dizziness and ataxia from streptomycin, even early in their treatment. Dihydrostreptomycin did not appear to cause this effect in the older patients, who tolerated it in the 1 gm. daily dosage very well. Accordingly, it seemed fitting that a study of a small series of cases in the older age group be undertaken with the hope that dizziness and ataxia might be prevented, and at the same time, by using a relatively small dosage and for a not too prolonged period, deafness would not occur in the group. Since there had been some reason to believe that the hydrochloride salt—or some impurity in this salt—was the offender and the cause of the deafness previously reported, it would be part of the protocol to use only the dihydrostreptomycin sulfate. The dosage used was 1 gm. daily and this was given intramuscularly in a single daily dose for four months.

OBSERVATIONS

It was observed early in the treatment, as it had been in the previous study when the dihydrostreptomycin sulfate (Merck) had been first acquired by the hospital and tested on patients in the last month of treatment, that there was very much less local tissue irritability from the sulfate than from either the dihydrostreptomycin hydrochloride or from the streptomycin calcium complex. The patients did not object to their daily injections. As the study progressed and no toxic manifestations at all developed, it appeared that the dihydrostreptomycin salt in the dosage given was a relatively nontoxic drug, although, because of past experience, it remained to be seen whether or not any deafness would develop post treatment. At the end of four months of therapy there had been no dizziness or ataxia in any of the 20 patients. Furthermore, there was no audiometric loss of 30 decibels or more at the frequencies of 128, 256, 512, 1,024, 2,048, and 4,096.

This group of patients has been observed for a period of four months to one year after the completion of the four months of treatment and no vestibular loss, or audiometric loss of significance, or any clinical deafness has been apparent in any of them.

SUMMARY

Twenty patients in the age group 45 and over were treated for four months with dihydrostreptomycin sulfate in a daily dosage of 1 gm. Four to twelve months after completion of treatment it can be stated that no symptoms of vestibular damage were noted at any time and there have been no audiometric changes of significance within the hearing range. There has been no clinical deafness.

In the dosage given and for the time period of the study (four months), dihydrostreptomycin sulfate appears relatively nontoxic. It seems to be better tolerated by patients over 45 years of age than is streptomycin in that no dizziness and ataxia have been observed when it has been used in 1 gm. daily dosage for no more than four months. In the absence of definite information at this time it is probably wiser not to use the dihydrostreptomycin sulfate for a period exceeding four months. If, however, the daily dosage is reduced to 1 gm. twice a week, it may conceivably be given with safety for six or eight months.

SUMARIO

El Sulfato de Dihidroestreptomicina en la Tuberculosis Pulmonar: Informe sobre la Toxicidad Dirigida al Octavo Par Craneal

Veinte enfermos fueron tratados con sulfato de dihidroestreptomicina a dosis de 1 gm. diario por espacio de cuatro meses. No se han notado ni sordera clínica ni alteraciones audiométricas de mayor importancia en ninguno de esos pacientes que han estado en observación por espacio de cuatro meses a un año después de terminar el tratamiento.

No ha habido deterioro vestibular ni ningún otro signo significativo de toxicidad.

Parece que, a la dosis administrada y durante el tiempo que abarcó el estudio, el sulfato de dihidroestreptomicina es una droga relativamente inocua y eficaz. Los AA. recomiendan el empleo del mismo a dosis diarias de 1 gm. durante un período que no exceda de cuatro meses en los sujetos de 45 años o más de edad que a menudo manifiestan con la estreptomicina vértigo, ataxia y deterioro vestibular. También lo recomiendan en las personas estreptomicino-hipersensibles. Los AA. no emplean la sal clorhidrato de dihidroestreptomicina ni favorecen su empleo. Por virtud de la mayor eficacia, gramo por gramo, de la estreptomicina, la recomiendan para la mayoría de los enfermos de menos de 45 años y para los que no revelan reacciones alérgicas a la misma.

RESUME

Le sulfate de dihydrostreptomycine dans la tuberculose pulmonaire: Rapport sur la toxicité exercée sur la huitième paire cranienne

Vingt malades ont été traités par le sulfate de dihydrostreptomycine à la dose de 1 gramme par jour pendant quatre mois. Il n'a pas été observé de surdité ou d'altération audiométrique de degré significatif chez un seul de ces malades, dont l'observation a été poursuivie pendant des périodes de quatre mois à un an après la fin du traitement.

Il ne s'est produit aucune perte de fonction du nerf vestibulaire et aucune manifestation appréciable de toxicité.

Aux doses données pendant la période de l'étude, le sulfate de dihydrostreptomycine paraît être un médicament relativement sans danger et efficace. Les auteurs recommandent son usage à la dose quotidienne de 1 gramme pendant une période ne dépassant pas quatre mois chez les malades âgés de 45 ans, ou plus, chez lesquels l'administration de streptomycine provoque souvent des vertiges, de l'ataxie et une perte de la fonction vesti-

bulaire. Il est également préconisé chez les sujets allergiques à la streptomycine. Les auteurs n'emploient pas le chlorhydrate de dihydrostreptomycine et n'en recommandent pas l'usage. En raison d'une plus grande efficacité, gramme pour gramme, de la streptomycine, la streptomycine est préconisée pour la majorité des malades de moins de 45 ans, et pour ceux qui ne présentent pas d'allergie à la streptomycine.

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AN EVALUATION OF FOUR METHODS OF COLLECTING AND MAILING GASTRIC WASHINGS FOR TUBERCLE BACILLI[1, 2]

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INTRODUCTION

The study of gastric contents from patients suspected of having pulmonary tuberculosis has long been a useful and accepted supplement for the establishment of the diagnosis. In 1898 Meunier (1) used the study of gastric contents as a means of diagnosing tuberculosis in children because of their habit of swallowing sputum. Thirty years later Armand-Delille (2) also demonstrated the value of gastric washings in establishing a diagnosis in infants when sputum examinations were unsatisfactory. Holm and Jensen (3) reviewed the use of gastric lavage for the decade 1931 to 1941 as a supplement to diagnosis and reported it as being especially serviceable in cases of ambulant patients in tuberculosis dispensaries.[4] At present, much consideration is being given to gastric lavage examinations for ex-sanatorium patients who may have little or no sputum or may show a reluctance to submit sputum specimens.

It is well known that tubercle bacilli are lost from gastric specimens if these are allowed to stand for twenty or more hours. This presents a serious problem to the many physicians who lack facilities for immediate examination after obtaining such specimens and must, therefore, send them through the mails to laboratories with proper facilities. As early as 1889 Straus and Wurtz (4) reported that the gastric juice of dogs destroyed the virulence of tubercle bacilli in eighteen to thirty-eight hours. Floyd and Page (5) found that, when tubercle bacilli were exposed to artificial gastric juice, the virulence of the microorganisms varied indirectly with the time of exposure.

The mechanism of action here is not known, but studies have been made which give various suggestions. Roper and Ordway (6) reported that one-tenth normal hydrochloric acid, even on continuous contact for forty hours, did not materially reduce the viability of tubercle bacilli. Bile also had no effect. Kramer (7) has suggested the possibility that either the gastric liapase acting in an acid medium or pepsin in the presence of hydrochloric acid may possibly be capable of destroying the pathogenicity of the bacilli. In the experience of Ames, Culver,

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[4] It is the considered belief of many observers (but not yet proved) that gastric specimens should be obtained promptly upon awaking and before the patient arises in order to avoid loss of bacilli by peristalsis after physical exercise begins.

and Nungester (8) a germicidal factor (G-1-r) may be present in gastric secretions and, as suggested by Piasecka-Zeyland and Zeyland (9), a substance in human saliva may inhibit the growth of tubercle bacilli.

In studies to determine the effect of time on the viability of tubercle bacilli in gastric contents, Kramer (7) determined that a period of ten to twenty-one hours at 37°C. rendered virulent tubercle bacilli nonpathogenic for guinea pigs, and he was unable to recover the microorganisms from their organs. Kabler and Lundholm (10) reported that 32 to 42 per cent of the specimens gave inhibition of growth when the specimen was held twenty-five hours at 4°C. Sprick and Towey (11), in a study of 33 gastric specimens, observed that, whereas 73 per cent were positive on an immediate examination, only 45 per cent were positive after twenty-four hours and only 21 per cent after forty-eight hours. The results of Holloway and Cummings (12) were in essential agreement.

In practically every instance, when gastric lavage specimens were mailed, the delay period was in excess of any acceptable time limit.

Due to such considerations as these, physicians are prone to refrain from mailing gastric lavage specimens to the laboratories, and laboratory personnel believe that their examinations are unsatisfactory because of false negatives. Therefore, efforts have been made to find a suitable method of mailing these specimens. These include the scheme proposed by Kabler and Lundholm (10) who reported that the addition of crystalline disodium phosphate to the specimen immediately following collection will enable the laboratory to obtain a considerably greater number of positive cultures from mailed specimens. Dilution of the specimen has also been suggested and is being used in Denmark (13).

The present study was undertaken to find a suitable medium with which to reduce the loss of tubercle bacilli in gastric contents, irrespective of whether it be caused by acid, enzyme, or both, or some other factor or combination of factors. The dilution and the neutralization methods previously tried are evaluated in this paper (*Experiments I and II*). *Experiment III* is a combination of *I and II*, and *Experiment IV* is a newly suggested method.

MATERIALS AND METHODS

The gastric specimens used in this study were from patients with suspected early tuberculosis whose sputum examinations had been repeatedly negative for tubercle bacilli, or from patients under close observation during or following treatment whose sputum examinations were similarly negative. Most of the specimens, therefore, contained relatively few tubercle bacilli. A satisfactory method of collecting and mailing specimens in which bacilli are scarce should be highly satisfactory for specimens which contain a large number of bacilli.

The present study comprised four evaluation experiments: *Experiment I*—a dilution method; *Experiment II*—a neutralization method; and *Experiments III and IV*—a dilution and neutralization method.

Experiment I: The gastric lavage was made following a twelve-hour fast. A Levin tube (12), cleaned, sterilized, and kept on ice continuously was used to collect the specimens. With a 60 ml. all-glass irrigating syringe having a catheter point, all available stomach con-

tents were withdrawn and placed in a labeled sterile 360 ml. screw-cap bottle. The syringe was re-connected to the tube with the plunger free, sterile distilled water was poured into the syringe and, upon replacing the plunger, the water was forced gently into the stomach. The diluted contents were then withdrawn and delivered into the 360 ml. bottle. This was continued until approximately 300 ml. sterile distilled water was used. The bottle was agitated vigorously and the contents divided. One half was treated immediately (see below) (within two hours) as a control and the other half was mailed to a point approximately seventy-five miles distant and promptly returned. The time of delay ranged from two to seven days, averaging approximately seventy-two hours. The examination procedure on return was the same as that used in the immediate examination.

Experiment II: The gastric lavage specimens were collected without dilution. A total volume of approximately 100 ml. was desired for all specimens in this experiment and, should the collected volume be less than 100 ml., sterile distilled water was added to obtain this desired volume. The specimen was agitated vigorously and one half of each specimen was placed in contact with crystalline disodium phosphate (Na_2HPO_4) and mailed. The other half was treated immediately as a routine specimen, this being a control. The bottle containing Na_2HPO_4 was prepared according to the procedure of Perry (14):

> Two hundred gm. anhydrous Na_2HPO_4 were dissolved in 300 ml. of distilled water by heating. Distilled water was added to make 500 ml. One and five-tenths (1.5) ml. of the hot solution were placed in each bottle. These were autoclaved with lids on loosely (15 pounds, 121°C. for twenty minutes), after which they were refrigerated over night. One and five-tenths (1.5) ml. of the solution is sufficient to neutralize approximately 45 ml. of N/10 HCl.

Experiment III: The procedure in *Experiment III* was the same as that described in *Experiment I* except that one half of the diluted gastric juice was placed in a mailing bottle which contained crystalline disodium phosphate (Na_2HPO_4) prepared from 4.25 ml. of the hot solution described above. The other half was treated immediately as a control specimen.

Experiment IV: The procedure in *Experiment IV* was the same as that described in *Experiment I*, except that two commercial "pHydrion"[5] capsules (pH 7.4) were emptied into the mailing bottle which subsequently contained one half of the diluted specimen. The other half was treated immediately as a control specimen.

All four experiments were handled as routine gastric specimens. The treatment was the same, whether immediate or delayed, and was as follows:

> Each specimen was agitated vigorously, divided between large centrifuge tubes, balanced, and centrifugalized at 3,000 r.p.m. for fifteen minutes.
>
> The supernatant fluid was poured into a splash-proof jar containing 5 per cent phenol. With a sterile Pasteur pipette, the sediment was pooled in one sterile screw-cap concentration tube. An equal volume of 4 per cent sodium hydroxide (NaOH) was added and the cap replaced tightly.
>
> The tubes were shaken for ten minutes in a paint conditioner, then centrifugalized at 3,000 r.p.m. for fifteen minutes. The supernatant fluid was discarded.
>
> The sediment was neutralized using a Pasteur pipette, with which two drops of 10 per cent HCl were added immediately to each tube in order to reduce the exposure time. One drop of phenol red was added with another pipette and the contents shaken vigorously. With another Pasteur pipette, 5 per cent HCl was added, drop by drop, until the end point was reached. Two per cent NaOH was used to back titrate until the first return of pink remained.

Cultures: Cultures were made by inoculating one half of the neutralized sediment equally on four tubes of Jensen's modification of Löwenstein's medium (15) by using a Pasteur pi-

[5] Commercial "pHydrion" capsules are made by Micro-essential Laboratories and are obtainable through most scientific supply houses.

pette. The water of condensation was poured off immediately preceding inoculation. The remaining half was placed in a sterile, cork-stoppered, 20 ml. animal inoculation bottle containing 1 ml. of sterile physiological saline. If a culture only was made, all of the neutralized sediment was inoculated equally on four tubes of Löwenstein medium. No culture was considered negative for tubercle bacilli until after six weeks' incubation at 37°C.

Animal inoculation: Animal inoculation was made by injecting the specimen subcutaneously into the right groin of a guinea pig which failed to show a cutaneous reaction to tuberculin. If the culture was positive for *M. tuberculosis* in four weeks, the corresponding guinea pig was tuberculin tested and, if found positive, autopsied immediately. All tuberculin-negative guinea pigs were held for six weeks, at which time the tuberculin test was repeated, after which the pigs were autopsied.

FIG. 1. Proposed outfit to be sent to those collecting gastric washings which must be mailed to the laboratory for tubercle bacilli examinations.

Mailing container: A kit of materials which may be sent to persons who wish to collect gastric specimens for mailing may be seen in figure 1.

RESULTS

Neutralization

Results of the neutralization procedure were not determined for *Experiments I and II*. In *Experiment III*, the initial reading averaged pH 3.49, the immediate neutralizing result averaged pH 7.82, and the delayed reading (after return to laboratory) averaged pH 7.52, giving an average drop in pH of 0.26. In *Experiment IV* the initial reading averaged pH 3.41, the immediate buffering result averaged pH 7.23, and the delayed reading averaged pH 7.10, with an average drop in pH of 0.13 and a maximum drop in pH of 0.3.

Concentrate Smear Examination

A study was made of 696 gastric lavage specimens, 36 of which showed acid-fast organisms by microscopic examination. All but 5 of the 36 yielded tubercle bacilli by culture or animal inoculation. Of the 5 patients from whom the 5 specimens with no tubercle bacilli were obtained, one had sputum positive for *M. tuberculosis* by culture and animal inoculation the following day; one had a positive culture from a gastric specimen collected three weeks later; 2 yielded acid-fast saprophytes on culture (animals inoculated from same specimens were negative); and one is still noninfectious after ten months of repeated culturing and animal inoculations.

In only 2 of the 5 patients were the microscopic examinations substantiated by later culture or animal inoculation. Therefore, in only 2 of 696 gastric lavage specimens (0.28 per cent) was the microscopic examination of value. This means, obviously, that culture and animal inoculations are far more efficient than microscopic examination in detecting tubercle bacilli in gastric contents and that the latter, requiring extensive time, is absolutely unnecessary.

Culture Without Animal Inoculation

In table 1 may be seen a comparison of the cultural results on immediate and delayed examinations for all four experiments. A continuous decrease in the percentage loss due to delay is shown in the order of *Experiments I, II, III, and IV*. The loss was 71.6 per cent in *Experiment I* and only 12.97 per cent in *Experiment IV*.

The results obtained on delayed examination with the dilution procedure are somewhat superior to the expectancy demonstrated by Sprick and Towey (11) on untreated delayed specimens. It may be well to mention that a higher percentage of positive results was obtained with the delayed examination during the colder days.

The procedure of neutralization with Na_2HPO_4 (*Experiment II*) seems far superior to dilution with distilled water alone. When the methods of *Experiments I* and *II* were combined, giving dilution plus neutralization with Na_2HPO_4 (*Experiment III*), results were still more favorable. When the dilution method was combined with buffering action of the "pHydrion" powder (*Experiment IV*), a procedure evolves which is superior to any one of the other three methods.

The data in table 2 are based on those specimens which were: (*1*) positive for tubercle bacilli on immediate and negative on delayed examination and (*2*) positive on delayed and negative on immediate examination. These data lend support to the statements drawn from table 1. The less the variation in the number and percentage of specimens found positive on the immediate and delayed examinations, the more nearly comparable should be the two procedures (immediate and delayed).

In the less satisfactory method (*Experiment I*) 72.8 per cent of the total positive specimens were demonstrated on the immediate examination only, with the delayed examination on these specimens being negative. In the more satisfactory

method (*Experiment IV*) there were only 28.9 per cent of the total positive specimens found on immediate examination only, and 18.4 per cent of the total positives were found only with the delayed examination.

TABLE 1

GASTRIC LAVAGE SPECIMENS EXAMINED AND PERCENTAGE OF ALL SPECIMENS POSITIVE FOR M. TUBERCULOSIS ON CULTURE WHICH WERE DETEPMINED BY IMMEDIATE AND DELAYED EXAMINATION, CLASSIFIED BY EXPERIMENT NUMBER AND METHOD

EXPERIMENT NUMBER AND METHOD	SPECIMENS EXAMINED			ALL POSITIVE SPECIMENS FOUND				PERCENTAGE LOSS IN POSITIVE DUE TO DELAY
	Total	Found positive		Positive on immediate examination		Positive on delayed* examination		
		Number	Per cent of total examined	Number	Per cent of total positives	Number	Per cent of total positives	
I. Dilution	246	92	37.4	88	95.4	25	27.3	71.6
II. Disodium phosphate	131	56	42.7	54	96.4	33	59.0	39.1
III. Dilution and disodium phosphate	146	43	29.5	37	86.4	28	66.1	24.3
IV. Dilution and buffer capsules	144	38	26.4	31	81.4	27	71.2	12.9

* Average delay, 72 hours.

TABLE 2

GASTRIC LAVAGE SPECIMENS POSITIVE FOR M. TUBERCULOSIS AS DETERMINED BY IMMEDIATE EXAMINATION ONLY AND BY DELAYED* EXAMINATION ONLY, CLASSIFIED BY EXPERIMENT NUMBER AND METHOD

EXPERIMENT NUMBER AND METHOD	SPECIMENS FOUND POSITIVE BY CULTURE				
	Total	Positive on immediate examination only		Positive on delayed* examination only	
		Number	Per cent of total	Number	Per cent of total
I. Dilution	92	67	72.8	4	4.3
II. Disodium phosphate	56	23	41.1	2	3.6
III. Dilution and disodium phosphate	43	15	34.1	6	13.6
IV. Dilution and buffer capsules	38	11	28.9	7	18.4

* Average delay, 72 hours.

Cultures with Animal Inoculation

The results are comparable with those of culture alone (table 1). Again there is demonstrated a continuous decrease in percentage loss due to delay in the order of the experiment number.

In *Experiment III* there was considerably less loss (10.3 per cent) in viable bacilli than in *Experiments I* and *II*.

In *Experiment IV*, 7.1 per cent more positive specimens were found on the

delayed examination than on the immediate examination. This indicates a greater sensitivity for the delayed examination and is superior to the other three methods in this laboratory.

In *Experiment IV* it appears that statistically the "delayed" examination is more satisfactory than "immediate." This is probably of little importance as Kayne and Hounslow (16), Sasano and his associates (17), and Robinson and Dunn (18) have demonstrated that culture and animal inoculation done simultaneously will give the highest percentage of positive results, with one of the tests being occasionally negative and the other positive. Comparison of the results shown in table 1 and table 3 confirms these findings. This being true, with two series of culture and animal inoculations as done in the present study with

TABLE 3

GASTRIC LAVAGE SPECIMENS EXAMINED AND PERCENTAGE OF ALL SPECIMENS POSITIVE FOR M. TUBERCULOSIS* WHICH WERE DETERMINED BY IMMEDIATE AND DELAYED EXAMINATION, CLASSIFIED BY EXPERIMENT NUMBER AND METHOD

EXPERIMENT NUMBER AND METHOD	SPECIMENS EXAMINED			ALL POSITIVE* SPECIMENS FOUND				PERCENTAGE LOSS OR GAIN IN POSITIVES* DUE TO DELAY
	Total	Found positive*		Positive on immediate examination		Positive on delayed† examination		
		Number	Per cent of total examined	Number	Per cent of total positives	Number	Per cent of total positives	
I. Dilution	209	95	45.4	91	95.8	33	34.8	−63.7
II. Disodium phosphate	100	48	48.0	45	93.7	27	56.2	−40.0
III. Dilution and disodium phosphate	124	49	39.4	39	79.4	35	71.6	−10.3
IV. Dilution and buffer capsules	108	36	33.3	28	77.8	30	83.5	+ 7.1

* Positive for *M. tuberculosis* by culture and/or animal inoculation.

† Average delay, 72 hours.

the immediate and delayed specimens, one would expect some minor variations as demonstrated, even if both had been performed with the same procedure at the same time.

The results in table 3 are on the whole much better in regard to total positive specimens than the results in table 1. This would be expected since animal inoculations as well as cultures were done on both the immediate and delayed examinations, thus giving a greater opportunity for demonstrating a larger number of positive specimens.

The data in table 4 (similar to table 2) lend support to the conclusion drawn from table 3.

In *Experiment I*, 65.6 per cent of all positive specimens were found on immediate examination only, with the delayed examination on the same specimen being negative for tubercle bacilli; whereas only 4.2 per cent of the total positive

specimens were found on immediate examination only. This wide variation in immediate and delayed examinations is narrowed in the order of the experiments. In *Experiment IV* only 16.7 per cent of the total positive specimens were found on the immediate examination only; the delayed examination produced 22.2 per cent of the total positive specimens, with the immediate examination on the same specimens being negative for tubercle bacilli.

TABLE 4

GASTRIC LAVAGE SPECIMENS POSITIVE FOR M. TUBERCULOSIS* DETERMINED BY IMMEDIATE EXAMINATION ONLY AND BY DELAYED† EXAMINATION ONLY, CLASSIFIED BY EXPERIMENT NUMBER AND METHOD

EXPERIMENT NUMBER AND METHOD	SPECIMENS FOUND POSITIVE BY CULTURE AND/OR ANIMAL INOCULATION				
	Total	Positive on immediate examination only		Positive on delayed† examination only	
		Number	Per cent of total	Number	Per cent of total
I. Dilution	95	62	65.6	4	4.2
II. Disodium phosphate	48	21	43.7	3	6.2
III. Dilution and disodium phosphate	49	14	28.5	10	20.4
IV. Dilution and buffer capsules	36	6	16.7	8	22.2

* Positive for *M. tuberculosis* by culture and/or animal inoculation.

† Average delay, 72 hours.

SUMMARY

Gastric contents tend to "lose" tubercle bacilli on standing. To obviate this and to make it safe to send such material through the mails without loss of viable tubercle bacilli due to the passage of time, a series of experiments were performed.

A comparison was made of the results obtained from examination of gastric specimens collected in various ways and subjected to (*1*) dilution, (*2*) neutralization, (*3*) dilution and neutralization, and (*4*) dilution and buffering.

Collection of the gastric specimen with approximately 300 ml. sterile distilled water and the addition of this lavage to the contents of two "pHydrion" capsules give a simple dilution and neutralization procedure entirely satisfactory for the preservation of viable tubercle bacilli in gastric lavage specimens mailed to the laboratory.

Collection of the specimen with approximately 300 ml. sterile distilled water and neutralization with crystalline disodium phosphate are only slightly inferior to the above method but are not so simple.

Neutralization of the gastric lavage specimen with disodium phosphate was found to be less satisfactory than either of the above procedures. Dilution alone proved the most unsatisfactory of the four methods studied.

A study of the examination of the concentrate smear and evaluation of the concentrate smear with culture and animal inoculation are given. The exami-

nation of the concentration smear is of little value, whether it be positive or negative for acid-fast bacilli.

Culture and animal inoculations performed with each specimen will give a significantly higher percentage of specimens positive for tubercle bacilli than if cultural procedures only are employed.

SUMARIO

Justipreciación de Cuatro Métodos para Recoger y Remitir por Correo Lavados Gástricos con Bacilos Tuberculosos

El contenido gástrico suele "perder" bacilos tuberculosos, si se deja en reposo. A fin de evitar esto y de asegurar el envío por correo de ese material sin pérdida de bacilos tuberculosos viables a causa del tiempo transcurrido, se proyectaron los experimentos descritos en este trabajo.

La comparación de los ejemplares gástricos recogidos de distintos modos y sometidos a la (*1*) dilución, (*2*) neutralización, (*3*) dilución y neutralización, y (*4*) dilución y amortiguamiento.

La colecta con unos 300 ml. de agua destilada estéril y la adición de ese lavado al contenido de dos cápsulas de "pHydrion" ofrece un sencillo procedimiento de dilución y neutralización absolutamente satisfactorio para la conservación de los bacilos tuberculosos viables en las muestras de lavados gástricos remitidas por correo al laboratorio.

La colecta con unos 300 ml. de agua destilada estéril y la neutralización con Na_2HPO_4 es apenas inferior al anterior (a), pero no tan sencillo.

La neutralización del ejemplar de lavado gástrico con Na_2HPO_4 resultó ser menos satisfactoria que uno u otro de los procedimientos anteriores. La mera dilución fué la menos satisfactoria de las cuatro técnicas estudiadas.

Preséntanse un estudio del examen de los frotes concentrados y una justipreciación de los resultados de los frotes concentrados con los de los cultivos e inoculaciones en animales. El examen de los frotes concentrados posee poco valor, ya sean positivos o negativos para bacilos ácidorresistentes.

Los cultivos y las inoculaciones en animales ejecutados con cada ejemplar darán un porcentaje significativamente mayor de ejemplares positivos para bacilos tuberculosos que el mero empleo de procedimientos de cultivo.

RESUME

Evaluation de quatre méthodes pour recueillir et expédier les liquides gastriques obtenus par lavage pour la recherche des bacilles tuberculeux

Le contenu gastrique tend à "perdre" rapidement ses bacilles tuberculeux. Pour obvier à cet inconvénient et pour permettre d'expédier ce matériel par la poste, sans perte des bacilles tuberculeux viables, que peut causer le délai apporté dans la recherche, les techniques décrites dans cet article ont été élaborées.

La comparaison de spécimens gastriques récoltés par des moyens différents et soumis aux procédés suivants: (*1*) dilution, (*2*) neutralisation, (*3*) dilution et neutralisation et (*4*) dilution avec addition d'un tampon.

Le prélèvement avec approximativement 300 ml. d'eau distillée stérile et addition de cette eau de lavage au contenu de deux capsules "pHydrion" est un procédé de dilution et de neutralisation, entièrement satisfaisant, pour la préservation des bacilles tuberculeux viables dans les spécimens de lavage gastrique expédiés au laboratoire.

Le prélèvement avec approximativement 300 ml. d'eau distillée stérile, suivi de neutralisation avec Na_2HPO_4 cristallisé, est légèrement moins favorable que le procédé ci-dessus (a) et n'est pas aussi simple.

Le procédé de neutralisation du spécimen de lavage gastrique par le Na_2HPO_4 a été

trouvé moins satisfaisant qu'aucun des deux procédés ci-dessus. La dilution seule s'est montrée la moins satisfaisante des quatre méthodes étudiées.

Une étude de l'examen du frottis de matériel concentré et l'évaluation de frottis par culture et par l'inoculation à l'animal est fournie. L'examen du frottis de matériel concentré est de peu de valeur, qu'il soit positif ou négatif, en ce qui concerne les bacilles acido-resistants.

La culture et l'inoculation à l'animal, effectuées avec chaque spécimen, fournira un pourcentage nettement plus élevé de spécimens positifs pour le bacille tuberculeux que la méthode des cultures seule.

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Case Reports

TULAREMIC LUNG ABSCESS[1]

A Case Report

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INTRODUCTION

The frequency of pulmonary involvement in tularemia has been emphasized by the numerous reported cases during the past two decades. In 1941, Blackford and Casey (1) reported 12 cases of their own and reviewed 150 other cases in the literature. In 1945, Stuart and Pullen (2) reported 15 additional cases; they found 253 previously reported cases at the time of their review. Heilman (8) in 1944 reported the successful treatment of tularemia in experimental animals with streptomycin, following which there had been numerous clinical reports (9–13) of the effectiveness of this antibiotic in the treatment of all forms of tularemia. Since 1949 the effectiveness of aureomycin (14, 15, 16) and chloramphenicol (Chloromycetin) (17) in the treatment of tularemia has also been well established.

Tularemic pneumonia occurs in approximately 15 per cent of all cases of tularemia, but Foshay (4) and others have reported pulmonary involvement in approximately 50 per cent of the typhoidal form of the disease. Although hematogenous dissemination obviously accounts for the pulmonary lesions in the ulceroglandular type of the disease, inhalation is probably the method of infection in the typhoidal form with pneumonic involvement. Reimann (5) has suggested that such inhalation could result from the *B. tularensis* being suspended in the air as dust from dried droppings or droplets from infected animals.

The pathology of tularemic pneumonia has been thoroughly reviewed and discussed by Blackford and Casey (1), Hunt (3), Lillie and Francis (6), Permar and Maclachlan (7), Stuart and Pullen (2), and many others. A lobular, caseous pneumonia, quite similar grossly to tuberculosis, is the usual finding. In addition, necrosis and cavitation are occasionally found but these are rarely prominent features of the disease. In fatal cases multiple lobes are usually involved.

Microscopically, the exudate is made up chiefly of mononuclear leukocytes. In cases with significant caseation and necrosis, there is also an acute inflammatory process involving the pulmonary arteries and veins, with thrombosis of these vessels.

Although necrosis and cavitation are often encountered among the autopsied cases (2, 3), it is unusual to encounter a definite lung abscess clinically in cases of tularemia. Therefore, the following case report is presented.

CASE REPORT

A 29-year-old white married farmer was admitted to the Medical College of Virginia Hospital on July 22, 1951 with the chief complaint of "I feel bad." He was apparently in good health until July 19, 1951 (three days before admission) when he began to complain of general malaise, weakness, low-backache, headache, and myalgia. The next day he suf-

[1] From the Department of Medicine, Medical College of Virginia, Richmond, Virginia.

fered anorexia and nausea. He also complained of pleuritic pain in his left chest anteriorly. On July 21, 1951, he was seen by his physician who prescribed a preparation of procaine penicillin and chloramphenicol. Symptoms became worse on the following day, so the patient was referred to the hospital. The patient had had contact with farm animals and ticks but with no wild animals. The past history was essentially negative.

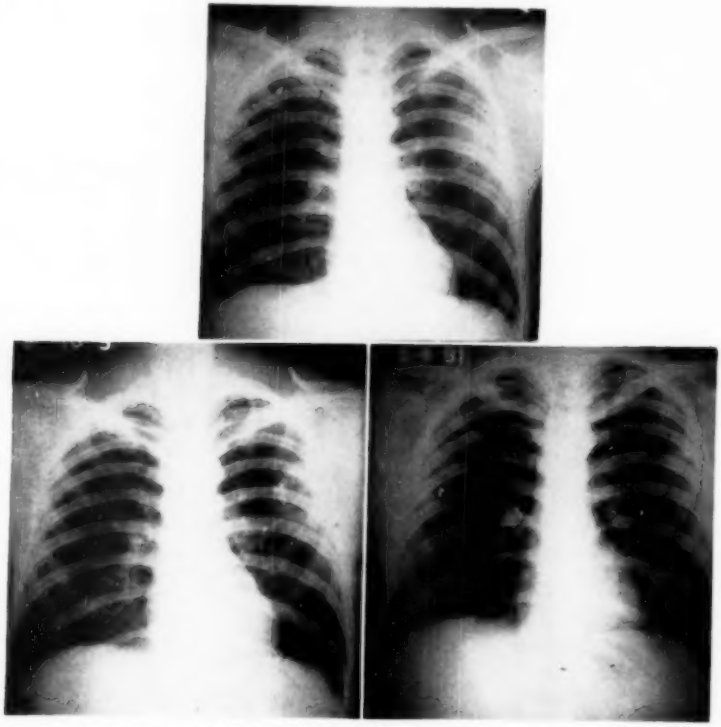

FIG. 1. Pneumonitis involving anterior segment of left upper lobe

FIG. 2. Large abscess in area of pneumonitis. Lateral film showed the abscess to be anterior.

FIG. 3. Complete disappearance of abscess and pneumonia

Physical examination on admission revealed a well-developed, well-nourished, acutely ill white male with a rectal temperature of 104°F.; pulse was 90 and respirations were 25 per minute; blood pressure was 120 systolic and 70 diastolic in mm. of mercury. Ear, eyes, nose, and throat showed no abnormalities except for moderate injection of conjunctiva and pharynx. The neck was not stiff, and there was no lymphadenopathy. The skin was normal. There were slight dullness, depressed breath sounds, and a few moist rales just beneath the angle of the left scapula. Examination of the heart, abdomen, and genitalia revealed no abnormalities; the neurologic examination was normal.

The urine on admission and in subsequent examinations was normal. The concentration

of hemoglobin was 16.5 gm. per 100 ml.; the total leukocyte count was 10,000, with 72 per cent polymorphonuclears, 1 per cent eosinophils, 23 per cent lymphocytes, and 4 per cent monocytes. A serologic test for syphilis was negative. Blood cultures were sterile. Sputum and gastric washings were negative for acid-fast bacilli on stained smear. Agglutinations for typhoid, paratyphoid, *Brucella*, *Proteus* X19, and tularemia were negative on the day of admission. A roentgenogram of the chest on admission to the hospital revealed a pneumonic density in the left second and third anterior interspaces (figure 1). Lateral film indicated involvement of the anterior segment of the left upper lobe.

The patient was started on 300,000 units of procaine penicillin fortified with 100,000 units of sodium penicillin every twelve hours, but his symptoms continued and the rectal temperature remained elevated between 101.2° and 105.8°F. He became confused and delirious. On the second hospital day, the patient was started on chloramphenicol (Chloromycetin), 500 mg. every four hours. After twenty-four hours of chloramphenicol, the temperature began to fall by gradual lysis. On July 27, 1951, after three days of chloramphenicol, the patient was afebrile and asymptomatic except for weakness. The drug was discontinued after the patient had been afebrile for two days. A repeated agglutination reaction on July 30, 1951 was positive for tularemia in a dilution of 1:160. This titer subsequently rose to 1:320 on August 2, 1951. The patient felt well and remained afebrile. On July 31, 1951, a chest film revealed a cavity measuring 3.5 cm. in diameter in the left upper lobe field at the level of the third anterior rib, with light mottling around the cavity (figure 2). A lateral film showed the cavity to be in the anterior segment of the left upper lobe. He had no pulmonary symptoms and felt perfectly well at this time. Chest roentgenograms on August 6 and August 10, 1951 showed definite, although slight, reduction in the size of the cavity. It was believed that the patient probably had a lung abscess due to tularemia, so chloramphenicol was again started on August 10, 1951. The next day the patient developed edema of the face, hands, and feet, with giant urticaria. The chloramphenicol was then discontinued. The allergic manifestations were controlled with epinephrine and antihistaminics. Since the patient was afebrile and asymptomatic but still showed roentgenographic evidence of a cavity, he was discharged on streptomycin therapy, 1 gm. three times a week for three weeks. On September 4, 1951 the patient was seen in the Chest Clinic. He was asymptomatic and felt well. A chest roentgenogram on that date was entirely normal (figure 3).

COMMENT

Although areas of necrosis are a frequent pathologic finding in cases of tularemic pneumonia, a large abscess cavity, as in the present case, is rather unusual except in patients dying of the disease. In view of the thin wall of the cavity and because of its size, it is likely that a "ball-valve" mechanism was present in the draining bronchus. It is probable that, following the development of caseation and necrosis, drainage of the liquefied material through a bronchus occurred and, because of a partial obstruction to the draining bronchus, a tension cavity resulted. By controlling the infection with chloramphenicol and streptomycin, the bronchial obstruction disappeared and the cavity closed. The absence of cough and expectoration during the period when the abscess was present is clinical evidence against the probability that the abscess was the result of excavation of lung tissue alone.

SUMMARY

A case of tularemic pneumonia is presented in which a large abscess developed within the area of pneumonia. The patient was treated initially with chloramphenicol, with prompt clinical improvement. Because of an apparent allergic reaction to chloramphenicol, the patient was later treated with streptomycin with complete disappearance of the lung abscess.

SUMARIO

Absceso Tularémico del Pulmón. Historia Clínica

En el caso de neumonía tularémica aquí presentado se formó un gran absceso en la zona neumónica. El enfermo fué tratado el principio con cloranfenicol, obteniéndose rápida mejoría clínica. Debido a aparente reacción alérgica al cloranfenicol, el tratamiento fué después con estreptomicina, que logró la desaparición total del absceso pulmonar.

RESUME

Abcès tularémique du poumon—Présentation d'un cas

Présentation d'un cas de pneumonie tularémique avec gros abcès situé dans le lobe intéressé. Le malade fut initialement traité par le chloramphénicol avec un résultat clinique favorable immédiat. Plus tard, en raison d'une réaction allergique paraissant due au chloramphénicol, le malade a été traité par la streptomycine et l'abcès a disparu complètement.

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Notes

SYNTHETIC ORGANIC BASES TESTED FOR INHIBITION OF TUBERCLE BACILLI

Various modifications of streptomycin are possible without loss of antibacterial activity, such as the reduction of the aldehyde group to produce dihydrostreptomycin, or the even more extensive change in the carbohydrate moiety found in mannosidostreptomycin. This fact implies that only certain of the structural features of streptomycin are essential for its activity. Furthermore, the inhibitory action of this drug is impeded by a variety of inorganic salts[1, 2, 3, 4] and has been shown by bactericidal tests to be extraordinarily sensitive to such interference[5]. As this effect is most readily understood in terms of competition for an intracellular receptor between the inorganic ions and an ionized group on the drug[6], the essential structures of streptomycin very likely include its ionizable groups (two guanido groups on the inositol ring and a methylamino group on the carbohydrate moiety). It therefore seemed possible that compounds having only a general resemblance to streptomycin, and in particular sharing its polybasic character, might show a similar antimicrobial action.

A cooperative project was consequently undertaken with Dr. Erich Mosettig and Dr. Everette May of the National Institutes of Health, with the aim of synthesizing and testing a series of polybasic, highly polar compounds designed to approach eventually the structure of streptomycin. Since these compounds were intended to resemble in their mode of action streptomycin, which is highly active against tubercle bacilli *in vitro*, they were tested only for this action.

The purpose of the present report is to record the results of tests on 64 compounds. These were all less active than streptomycin *in vitro*, and the most active one was considerably more toxic. These compounds therefore appear to offer no chemotherapeutic promise against tuberculosis. It was unfortunately not found possible to carry the syntheses to the point where the spatial relations between the basic groups would resemble closely those present in streptomycin.

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TABLE 1

COMPOUNDS TESTED FOR INHIBITION OF GROWTH OF TUBERCLE BACILLUS H37Rv IN TWEEN-ALBUMIN LIQUID MEDIUM

NIH NUMBER	NAME	INHIBITION BY 10 γ PER ML.
1338W	Quinoline-4-N,N-di-*n*-butylcarboxyamidine hydrochloride	None
2958	*p*-Nitrophenylbiguanide hydrochloride	None
2978	*p*-Nitrophenylguanidine	None
2979	*p*-Aminophenylguanidine hydrochloride	None
2980	*p*-Guanidophenylguanidine dihydrochloride	None
2981	1-(1-*p*-Biguanido)phenylbiguanide tetrahydrochloride. ½ H_2O	None
2982	*p*-Aminophenylbiguanide trihydrochloride. 2 H_2O	None
3436W	*o*-Chlorophenyl-dibutylamidine hydrochloride	None
3437W	Styryldibutylamidine hydrochloride	None
3438W	*p*-Methoxyphenyl-dibutylamidine hydrochloride	None
3439W	2,5-Dimethoxyphenyl-dibutylamidine hydrochloride	None
3440W	Mesityldibutylamidine hydrochloride	None
3441W	N-Benzyl-N′-phenylcarbimidopiperazine dihydrochloride	None
3489	D,L-Pentahydroxycyclohexylamine hydrochloride	None
3517	Semicarbazone of D,L-*epi*-inosose	None
3518	D,L-1-Pentahydroxycyclohexylsemicarbazide	None
3528W	*o*-Tolyl-N,N-dibutylamidine hydrochloride	Partial
3529W	*p*-Chlorophenyl-N,N-dibutylamidine hydrochloride	Partial
3530W	Phenyl-N,N-dibenzylamidine hydrochloride	None
3531W	4-Quinolyl-N,N-diheptylamidine hydrochloride	Complete
3532W	9-Acridyl-N,N-dibutylamidine hydrochloride	Partial
3533W	*m*-Methoxyphenyl-N,N-dibutylamidine hydrochloride	None
3534W	2,6-Dimethoxyphenyl-N,N-dibutylamidine hydrochloride	None
3535W	3,4-Dimethoxyphenyl-N,N-dibutylamidine hydrochloride	None
3582	*rac.*-*epi*-Inosose oxime	None
3583	*rac.*-Dimethylaminopentahydroxycyclohexane hydrochloride	None
3584	*N*-Glucoside of *rac.*-pentahydroxycyclohexylamine	None
3631	Tetraacetyl-β-2,4-diaminophenyl-D-glucoside dihydrochloride	None
3634	β-*o*-Aminophenyl-D-glucoside	None
3640	β-2,4-Diaminophenyl-D-glucoside	None
3641	Pentahydroxyaminocyclohexane(Inosamine-SA)hydrochloride. 1 H_2O	None
3646	β-*p*-aminophenyl-D-glucoside	None
3752	Tetraacetyl-β-2,4-diguanidinophenyl-D-glucoside sulfate	None
3769	β-2,4-Diguanidinophenyl-D-glucoside sulfate	None
3773	β-*m*-Aminophenyl-D-glucoside	None
3776	*o*-Tetraacetyl-*m*-(1-guanidinophenyl)-D-glucoside hydrochloride	None
3783	β-*m*-Guanidinophenyl-D-glucoside	None
3786	Tetraacetyl-β-*p*-guanidinophenyl-D-glucoside hydrochloride	None
3787	β-*p*-Guanidinophenyl-D-glucoside hydrochloride	None
3788	Thiosemicarbazone of D, L-*epi*-inosose	None
3789	Tetraacetyl-*o*-β-guanidinophenyl-D-glucoside hydrochloride	None
3842	β-*o*-Guanidinophenyl-D-glucoside	None
3844	N,N-Dimethyl-inosamine SA hydrochloride	Partial
3845	*Scyllo*-inosose thiosemicarbazone	None
3898	*p*-Nitrophenyl 3,4,6-triacetyl-β-D-glucosaminide hydrochloride	None

TABLE 1—*Continued*

NIH NUMBER	NAME	INHIBITION BY 10 γ/ML.
3899	2,4-Diguanidinophenyl 3,4,6-triacetyl-β-D-glucosaminide trihydrochloride	None
3913	2,4-Diaminophenyl-β-D-glucosaminide	None
3924	2,4-Diaminophenyl 3,4,6-triacetyl-β-D-glucosaminide	None
4086	2,4-Diguanidinophenyl-β-D-glucosaminide sulfate. 4 H_2O	None
4092	*p*-Guanidinophenyl-β-D-glucosaminide	None
4124W	4-Quinolyl-*N*,*N*-dihexylamidine hydrochloride	Partial
4125W	4-Quinolyl-*N*,*N*-dioctylamidine hydrochloride	Partial
4208	1-[(1-Piperidino)methyl]-inositol	None
4209	1-(1-Morpholino)-methylinositol	None
4210	4-Guanidino-3-pentadecylphenyl-β-D-glucoside hydrochloride	Partial
4211	1-[(N-2-hydroxyethylamino)methyl]-inositol	None
4215	N-Acetyl-5-aminomethyl-*meso*-inositol	None
4311	N-Acetyl-5-benzylaminomethyl-*meso*-inositol	None
4312	5-Benzylaminomethyl-*meso*-inositol hydrochloride	None
4325	5-Ethanolaminomethyl-*meso*-inositol hydrochloride	None
4382	6-Guanidino-3-pentadecylphenyl-1-β-D-glucoside	Partial
4545	*p*-Aminobenzoyl-*meso*-inositol	None
4546	*p*-Aminobenzoyl-*epi*-inositol	None
4600	*p*-Aminosalicylyl-*meso*-inositol	None

EXPERIMENTAL

Materials and Methods

The compounds noted in table 1 by the letter W following the NIH number were furnished to Dr. Mosettig through the kindness of the Wellcome Research Laboratories; the rest were synthesized at the National Institutes of Health.[7-11]

The culture medium employed[12] contained mineral salts, 0.2 per cent enzymatic casein hydrolysate (N-Z-Amine, Type B, Sheffield); 0.05 per cent Tween 80,[13] 0.2 per cent glucose, and 0.2 per cent heated serum albumin. The experiments were carried out with 10^{-5} and 10^{-6} ml. inocula of seven-day cultures of a virulent human tubercle bacillus, strain H37Rv, added to 5 ml. of medium in metal-capped 25 mm. test tubes. The compounds under investigation, filter-sterilized through sintered glass (Corning UF) in aqueous solution, were tested in final concentrations of 100, 10, and 1 γ per ml. The tubes were examined visually

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[12] DUBOS, R. J., AND DAVIS, B. D.: Factors affecting the growth of tubercle bacilli in liquid media, J. Exper. Med., 1946, *83*, 409.

[13] Proprietary name of the polyoxyethylene derivative of Sorbitan monooleate, Atlas Powder Co., Wilmington, Delaware.

for growth after seven and fourteen days of incubation at 37°C. As an inhibitory control of known efficacy, streptomycin was tested in each experiment at concentrations of 1, 0.3, and 0.1 γ per ml.

RESULTS

The control tubes without drug showed turbid growth of the 10^{-3} but not the 10^{-6} inoculum at seven days; at fourteen days both showed heavy growth. The streptomycin control tubes showed complete inhibition of growth of both inocula at 0.3 γ per ml. and decreased or delayed turbidity at 0.1 γ per ml.

The synthetic compounds tested are listed in table 1. Since the results were not encouraging, the details are not presented. Most of the compounds were not inhibitory even at 100 γ per ml.; some were inhibitory at that concentration, but only partly or not at all at 10 γ per ml. Only one compound, 4-quinolyl-N,N-diheptylamidine hydrochloride, was completely inhibitory at 10 γ per ml.; it also showed slight effect at 1 γ per ml. This compound was therefore studied further.

In view of the observed binding of other chemotherapeutics by proteins, especially serum albumin[14], the effect of this substance on the activity of this amidine compound was investigated. In two experiments, employing the methods described above, the addition of 1.0 per cent serum albumin did not interfere with the inhibition.

Toxicity studies were carried out in four- to six-week-old male mice, line 1 dba, averaging 20 gm. in weight. Daily subcutaneous injection of 500 to 1,500 γ of the drug produced death in nine to eighteen days, with toxicity prior to death indicated by weight loss, roughness of hair, and cutaneous ulcerations and sometimes subcutaneous necrosis at the sites of inoculation. Autopsy showed subcutaneous hemorrhages and adhesions but no gross evidence of organ damage. Daily injection of 125 or 250 γ for twenty-nine to thirty-one days was not fatal but did cause toxic symptoms and cutaneous ulceration.

A preliminary chemotherapeutic test with strain H37Rv, administered to mice via the tail vein, showed no protection and even more extensive pulmonary lesions in the animals receiving the drug (125 γ daily) than in control animals without the drug. A simultaneous group receiving streptomycin (500 γ daily) showed no toxicity, but also unexpectedly showed no chemotherapeutic effect. The chemotherapeutic test therefore cannot be considered decisive. However, in view of the obvious toxicity of the compound, further investigation of it did not seem warranted.

SUMMARY

Screening of 64 basic compounds, designed to have some resemblance to streptomycin, yielded only one (4-quinolyl-N,N-diheptylamidine hydrochloride) that prevented growth of tubercle bacilli in Tween-albumin medium in a concentration of 10 γ per ml. This compound was approximately one-tenth as active as streptomycin *in vitro* and was much more toxic in mice.

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December 6, 1951

[14] DAVIS, B. D.: The binding of chemotherapeutic agents to proteins and its effect on their distribution and activity, in *Evaluation of Chemotherapeutic Agents*, edited by C. M. MacLeod, Columbia University Press, 1949, p. 44.

A METHOD FOR THE DETERMINATION OF IN VITRO SENSITIVITY OF TUBERCLE BACILLI TO PYRAZINAMIDE (ALDINAMIDE[1])

In the clinical trial of pyrazinamide (Aldinamide)[2] some experience has been gained regarding the emergence of drug-resistant strains of tubercle bacilli. Because of the therapeutic potentialities of this drug, a description of the method used for the determination of pyrazinamide susceptibility seems desirable.

Dubos liquid medium[3] was prepared and pyrazinamide was added in four different concentrations after autoclaving the aqueous solution at 15 pounds for twenty minutes.

TABLE 1

STANDARD	COLORIMETER READING	BACILLI PER MILLILITER
0	not readable	8,000,000 and less
1	94	13,600,000
2	75	48,000,000
3	50	102,000,000
4	14	408,000,000

TABLE 2

STRAIN S.P. 2405/50	PATIENT T. BEFORE TREATMENT	MICROGRAMS PER MILLILITER	GROWTH INDICES AFTER	
			1 week	2 weeks
		0	1.0	2.0
		1.0	0.8	1.6
		10.0	0.6	1.2
		100.0	0	0
		1,000.0	0	0

The inoculum was prepared from primary cultures of tubercle bacilli on combination egg medium. Approximately three loopfuls were ground in sterile m/15 Na_2HPO_4. The strength of the suspension was determined by counting the number of bacilli contained in 0.005 cc. of the fluid placed on a slide marked with a square centimeter. The suspension was diluted to a strength of 8,000,000 bacilli per milliliter.

Five turbidimetric barium sulfate standards were prepared, and the reading on the Leitz colorimeter for each of these standards was used to determine the number of organisms per milliliter causing the same degree of turbidity (table 1).

Growth of the tubercle bacilli in the cultures was measured by comparing the turbidity of the medium with that of the five standards and by assigning to each tube the value of the standard nearest to it in degree of turbidity. Five tubes were used for each drug concentration (0, 1.0, 10.0, 100.0, and 1,000.0 γ per ml.). The average value found for five

[1] Proprietary name of the American Cyanamid Company.

[2] YEAGER, R. L., MUNROE, W. G. C., AND DESSAU, F. I.: Pyrazinamide (Aldinamide) in the treatment of pulmonary tuberculosis, Am. Rev. Tuberc., 1952, *65*, 523.

[3] DUBOS, R. J., AND MIDDLEBROOK, G.: Media for tubercle bacilli, Am. Rev. Tuberc., 1947, *56*, 334.

tubes was called the growth index. It was determined one and two weeks after inoculation. An example of how to tabulate the results of this method of evaluation is given in table 2.

The same case illustrates the degree of drug susceptibility typical for strains isolated from untreated patients. All such strains of tubercle bacilli were completely inhibited for a fourteen-day period by 100 and 1,000 γ of pyrazinamide per ml. of medium. There was partial inhibition at 1 and 10 γ of pyrazinamide per ml., which varied in degree from strain to strain. There was considerable variation of the values of the indices from one experiment and from one strain to the other. Loss of susceptibility in strains from treated patients seemed gradual. Growth at 1,000 γ per ml. two weeks after inoculation was common for strains of *M. tuberculosis* isolated from patients who had received two months of treatment with pyrazinamide.

Despite the fact that important information was obtained by the use of this method, it has shortcomings which make the development of other methods desirable. Evaporation of water tends to cause false nephelometric values. The need for a very large inoculum precludes the use of primary cultures. For these reasons, work is in progress now to adapt primary culture on solid media to the determination of the pyrazinamide susceptibility of tubercle bacilli.

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February 18, 1952

DISCUSSION ON TREATMENT OF TUBERCULOUS MENINGITIS AND SURVIVAL OF BACILLI IN TUBERCULOUS LESIONS

At the request of the Editors, Dr. Dubos has kindly consented to incorporate the substance of his extemporaneous remarks at the Conference on Chemotherapy of Tuberculosis of the Veterans Administration, held in January 1952, in the form of the present communication which is being presented here through the courtesy of Dr. Arthur Walker. The full text will be published in the Transactions of the Eleventh Veterans-Army-Navy Conference on Chemotherapy of Tuberculosis, January 17–20, 1952.

I shall depart from the paper which I had prepared for the meeting this afternoon, and shall present instead a few observations which may throw some light on two of the topics discussed today, namely, (a) the treatment of tuberculous meningitis, (b) the statement by Dr. D'Esopo that bacilli may die in certain tuberculous lesions even without the benefit of chemotherapy.

I shall single out three points from the discussion on meningitis: (*1*) The number of bacilli in the cerebrospinal fluid is always small; (*2*) it is imperative to continue streptomycin therapy for extremely long periods of time if one wishes to obtain a permanent cure; (*3*) granted that several of you do not look with favor upon the intrathecal administration of tuberculin, it is the consensus of the few investigators who have tried this method that it enhances the therapeutic effectiveness of streptomycin.

Although it is acknowledged by everyone that there are only few bacilli in the cerebrospinal fluid of the patient suffering from tuberculous meningitis, this statement does not describe adequately the important aspects of the bacteriology of the disease. The bacilli which are responsible for the pathological alterations and symptoms are not in the cerebrospinal fluid but in the lesions, where they are more or less protected within a fibrinous wall or matrix. It is probable that small numbers of bacilli are now and then released from these depots into the cerebrospinal fluid and can thus initiate new foci of disease. The necessity of maintaining chemotherapy for prolonged periods of time to prevent relapses may well be due to the fact that the fibrinous lesions are not readily penetrated by streptomycin.

Intrathecal administration of tuberculin brings about an intense inflammatory reaction at the site of the lesions. The inflammatory cells bring to the lesion a supply of proteolytic enzymes which may exert some destructive action on the fibrin. Furthermore, it is possible that the allergic reaction brings about an activation of the normally inactive protease of the serum and that this enzyme can also attack the fibrin. If these assumptions are correct, one may postulate that proteolytic attack on the fibrinous lesion brings about the release of the bacilli into the cerebrospinal fluid, or at least makes them more available for drug action. Although there is, to my knowledge, no published proof of an increase in proteolytic activity following intrathecal administration of purified protein derivative (PPD), some indirect evidence that such an increase occurs is found in the reports of the Oxford group in England. For example, they have observed at autopsy of some of their patients complete resolution of the fibrinous lesion. In addition, Dr. Honor Smith has also stated that a shower of bacilli in the cerebrospinal fluid sometimes follows the administration of PPD even in the presence of streptomycin.

If it is true that fibrinolysis facilitates the antimicrobial effect of the drug, it would appear logical that a similar beneficial effect might be obtained by intrathecal administration of proteolytic enzymes. In fact, streptokinase has been used for this purpose, but with indifferent results. This failure, however, does not settle the issue. Although streptokinase

is often referred to as a "fibrinolytic enzyme," it does not, per se, possess fibrinolytic activity. Streptokinase does nothing more than activate an enzyme-precursor which is normally present in serum in an inactive form; it is this activated serum factor which brings about fibrinolysis. Furthermore, the work of Dr. Tillett and his colleagues has shown that the enzyme-precursor is not overly abundant in normal tissues. It appears possible, therefore, that, where streptokinase has been used, the serum precursor may have been the limiting factor and that not sufficient active protease was present to permit effective fibrinolysis. For a contrary view, see Fletcher, A. P., Lancet, 1951, *2*, 290.

I shall now turn to Dr. D'Esopo's remarks. He has pointed out that, even without antimicrobial therapy, tubercle bacilli die spontaneously in closed caseous areas. It is not likely that this tuberculocidal effect is due to the activity of living phagocytic cells, since these cells can hardly function or even survive in necrotic areas. The damage to the bacilli cannot be traced either to antibody, since bacillary multiplication becomes intense again when plasma (and therefore antibody if present) repenetrates the lesion during liquefaction. Furthermore, it is well recognized that the infection can progress in certain parts of the body at the same time that the numbers of bacilli are decreasing in the closed caseous areas in the same individual. From these various considerations it appears certain that the death of bacilli is the result of the physico-chemical environment prevailing locally in the closed caseous lesion.

Since tubercle bacilli are, as far as is known, obligatory aerobes, it has been generally assumed that oxygen deprivation is the most important factor in checking their growth and causing their death in the closed lesion. It seems to me, however, that the amount of oxygen *in solution* in the tissue fluids is usually sufficient to allow for bacillary survival and even for some growth. In addition to its direct effect on the bacilli, however, low oxygen tension may have indirect effects by altering the metabolism of tissue cells and thereby modifying the environment in which the bacilli are situated. It is very likely, for example, that the tissue cells surrounding the tuberculous lesion have a predominantly glycolytic metabolism and accumulate locally very large concentrations of lactic acid. Probably because of this fact, the lesion is definitely acidic, but it is doubtful that it is ever sufficiently acidic to exert a deleterious effect on tubercle bacilli merely on account of the low pH. The concentration of organic acids, however, may have effects which are independent of pH level. It has recently been found, for example, that sodium lactate in the concentrations prevailing around caseous areas is capable of inhibiting the growth of many strains of tubercle bacilli even at neutral reactions.

It is known furthermore, that most long-chain fatty acids (especially the C_{10}, C_{12}, C_{14}, C_{16}, and C_{18} acids) are powerful antimycobacterial agents *in vitro*. These long-chain fatty acids are released by lipolytic enzymes during necrosis and there is no doubt that they are abundant in caseous material. As their antibacterial effect is the greater the more acidic the environment, it is not unlikely that they contribute to rendering the closed caseous area unfavorable for bacillary growth. Finally, experiments now in progress in our laboratory are revealing that long-chain fatty acids and lactic acid in low concentrations do become actually bactericidal when tubercle bacilli are exposed to them under the semi-anaerobic conditions that probably prevail in many lesions.

It has long been suspected that certain substances exist in normal tissues and in body fluids which exert a bacteriostatic and bactericidal effect on tubercle bacilli. I shall merely state that my colleague, Dr. James G. Hirsch, and I have devoted much effort during the past two years to separating these antimycobacterial agents and identifying their chemi-

cal nature. In brief, we have recognized the antimycobacterial activity of four groups of basic substances:

(a) The basic proteins, protamines and histones. These substances occur in cell nuclei and are liberated from their union with nucleic acid as a result of necrosis.

(b) The enzyme lysozyme. This enzyme is abundantly present in leukocytic cells and is known to accumulate in an active form in inflammatory areas. Let me mention in passing that lysozyme is also very abundant in egg white. It is probably to avoid its inhibitory effect on bacterial growth that bacteriologists have empirically eliminated as much of possible of the egg white in the preparation of egg media.

(c) The polyamines spermine and spermidine. These substances occur in certain tissues, often in large amount. In concentrations of the order of 1:100,000 they inhibit *in vitro* the multiplication of even large inocula of tubercle bacilli. In fact, bacilli maintained in contact with spermine or spermidine for two to three days are no longer capable of growing in ordinary culture media. It is a peculiar fact that neither spermine nor spermidine inhibits growth of the bacilli in synthetic media; their inhibitory power depends upon the presence in the medium of some other substance of unknown nature which occurs in bovine serum, bovine plasma fraction V, and aqueous extracts of certain animal tissues, but not in crystalline serum albumin.

(d) A substance, as yet unidentified, which has been separated from thymus and which exhibits a high antimycobacterial activity not dependent upon the presence of serum in the test system.

The facts which I have outlined make it clear that there prevails in tissues, and particularly in inflammatory and necrotic areas, a biochemical environment which is potentially capable of inhibiting the multiplication of tubercle bacilli and even of interfering with their survival.

It would be tempting to attempt to analyze what effects the changes which are known to occur in this environment under certain pathological conditions have on the course of the disease. Thus one may ask whether the enhancement of infection caused by cortisone or corticotropin is not due in part to the decrease in the production or in the local accumulation of tuberculostatic substances; this might well be a secondary result of the inhibition of the inflammatory reaction by these hormones. It seems possible also that alterations in the metabolism of leukocytes, for example in their relative glycolytic and oxidative activities, may change the behavior of these cells toward tubercle bacilli. This might possibly be of significance in the greater susceptibility to tuberculosis of the uncontrolled diabetic patient. Unfortunately, these speculations will not be amenable to experimental testing until techniques are developed to test *in vivo* the activity of the antimicrobial agencies revealed by *in vitro* tests. Moreover, new types of observations are necessary before it becomes possible to evaluate the real significance of tuberculostatic effects on the evolution of the tuberculous process. We are in need of new bacteriologic techniques to determine not only the viability of the tubercle bacilli as they occur in lesions but also their physiologic activity. Despite current belief, the guinea pig is not so susceptible to tuberculosis that it will always develop progressive disease following injection of "a few" living bacilli; and it is not known whether aging of the bacteria or "adsorption" on their surface of certain tissue constituents may not decrease their ability to establish infection or to initiate growth *in vitro*. In many fields of bacteriology, it has often proved difficult to differentiate clearly between "life" and "death" of bacterial cultures. It would be odd if the same difficulty did not hold true for tubercle bacilli.

But granted all these technical difficulties, it appears justifiable to believe that the local physiochemical environment prevailing in and around the tuberculous lesion is of paramount importance in determining the course of the infectious process by affecting the chemical structure of the lesion, the physiological activities of the tissue cells, and the survival and rate of multiplication of the bacilli.

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January 28, 1952

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To the Editors of The American Review of Tuberculosis:

BCG vaccine has been produced weekly in this laboratory for sixteen consecutive years and has given consistent results culturally in animals and in humans (*see also* Birkhaug, K., Am. Rev. Tuberc., 1949, *59*, 567). The fact that results, as far as tuberculin conversion is concerned, in a series of newborn infants ten years ago were identical with those of the present study further attests the uniform standardization of the vaccine.

In working with freeze-dried preparations, we noted that there was a lag phase in the growth cycle of BCG, so that six weeks' incubation was necessary. We have also shown that, whereas the conversion of the tuberculin reaction in infants vaccinated at two days of age was twenty-eight days for the fresh vaccine, it was fifty-eight days for the dried; albeit, at the end of a year practically all were strongly positive to the patch test. Our impressions are that both the dried and the stored fresh organisms go into a resting phase (Rosenthal, S. R., Am. Rev. Tuberc., 1952, *65*, 344). In this phase, respiration is reduced, and this accounts for the reduced oxygen uptake noted in stored vaccine or the delay in reducing methylene blue (Rosenthal, S. R., Haegen, B., and Bingenheimer, J., to be published).

Several years ago, a method of standardization of BCG vaccine was adopted which is, in the main, similar to those of the Laboratory of Biologic Control of the National Institutes of Health. The details are to appear in the *Annales de l' Institut Pasteur*. Briefly, the method and results obtained thereof are: (*1*) *Viability*—Serial dilutions in Youmans' media plus 0.5 per cent albumin gave growth in the 10^{-8} to 10^{-10} tube. Similar results were obtained if vaccine was stored at 4°C. for four or five weeks (Williston, E., and Rosenthal, S. R., to be published). (*2*) *Tuberculin conversion*—One puncture through a drop of vaccine gave positive reactions in guinea pigs one week later in 50 per cent of the animals or better. In newborn infants, using the multiple puncture disc (Rosenthal, S. R., J. Pediat., 1950, *36*, 399), 100 per cent were positive twenty-eight days following vaccination for the fresh and fifty-eight days for the dried. (*3*) *Virulence*—The mother culture (veil of potato culture) from which vaccine is finally made is inoculated intraperitoneally into guinea pigs (10 mg.). The latter are sacrificed at the time the fresh vaccine is released, which is usually three weeks later. In this way, some indication of virulence may be had before releasing the vaccine. There should be no gross lesions outside of the abdominal cavity. The vaccine itself is also inoculated intraperitoneally (5 to 10 mg.). The animals are killed one and one-half to twelve months later. There should be no progressive pathology. (*4*) *Sterility*—Tests are made in thioglycollate, broth, and agar media. The fresh vaccine may be released after forty-eight hours, but sterility tests are continued for seven days. Incubation is at 32° to 35°C. (*5*) *Morphology*—By Ziehl-Neelsen staining and by phase microscopy, typical curved rods should be seen. They should be acid-fast.

The dried vaccine is released when sterility, viability, tuberculin conversion in animal and man, morphology, and preliminary virulence studies are completed. (This usually is three months after preparation of the vaccine.)

The Laboratory of the University of Illinois and Research Foundation has been licensed to produce and distribute BCG vaccine (freshly prepared and dried) by the Federal Security Agency through the Laboratory of Biologics Control of the National Institutes of Health.

Sol Roy Rosenthal

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December 13, 1951

Books

ATLANTE ANATOMO-RADIOLOGICO DELLA TUBERCULOSI POLMONARE E MALATTIE NON TUBERCOLARI DELL'APPARATO RESPIRATORIO. Second edition. By Attilio Amondec-Sorini, Luigi Pigorini, and Gilberto Scorpati. Instituto Carlo Forlanini, Rome, Italy. Pp. 339 with photographic plates, schematic colored drawings, and X-ray reproductions, Societa Edifrice Universo, Roma, 1950, Price $15.00

This 10.5-by 13.5-inch volume, printed on heavy glossy paper is divided into four parts. Part I, 26 pages, deals with the roentgenographic approach to the study of the lung and with the technique of the autopsy and in anatomic preparations of the thoracic organs. Part II, 38 pages, deals with (*1*) the radiologic and anatomic picture of the normal thorax including the more common anomalies; (*2*) the chief types of lesions produced by the tubercle bacillus, including an excellent demonstration of formation of the various types of cavities. Part III, 158 pages, includes discussion and anatomic and radiologic demonstrations of primary, miliary, and "apical" tuberculosis; "infiltrates," "ulcerative lobitis;" and tuberculous pneumonias, chronic phthisis, pulmonary bronchial and vascular and pleural lesions. It includes a brief discussion of antimicrobial therapy of pulmonary tuberculosis and several illustrative plates. It concludes with a section on collapse therapy. Part IV, 41 pages, consists of a brief but concise and fairly inclusive section on nontuberculous pulmonary diseases including bronchiectasis, cystic disease, pulmonary lesions and gangrene, pneumonconiosis, and tumors.

The outstanding feature of the Atlas is the reproduction, by means of strikingly clear, colored photographic plates, of anatomic material. In most instances each specimen is accompanied by schematic, partly colored drawings of the same specimen for the purpose of labeling structures and lesions. Little less striking are most of the photographic reproductions of illustrative roentgenograms.

The text is well organized and covers adequately and accurately the topics under discussion.

As a reference guide for students and for the purpose of demonstration by the teacher, this Atlas is of considerable value and is deemed a notable contribution to the reference literature on pulmonary diseases.

ALPHONSE TIMPANELLI
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ATLAS OF OPERATIVE THORACOSCOPY. By Stanko Dujmušić. Pp. 216, "Medicinska Knjiga," Belgrade, Yugaslavia, 1951.

In his monograph, Dr. Dujmušić followed the old Chinese proverb that "one picture is worth more than ten thousand words." The superb illustrations reproduce with surprising accuracy the various conditions seen through a thoracoscope and present the best exhibit of this type that has ever been published. Because only technical problems are considered, both some experience and supplemental information are necessary to make this book of practical value. In addition there may well be a difference of opinion whether some of the extensive technical procedures described are ever indicated.

This book is recommended for an institution in which thoracic surgery is done.

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American Trudeau Society

Medical Section of the National Tuberculosis Association

The Problem of the So-Called "Good Chronic" Case of Pulmonary Tuberculosis

A Statement Prepared by the Committee on Therapy

I. DEFINITION

An exact definition is difficult. Many people object to the term "Good Chronic" on grounds that no chronic tuberculosis is good. While this objection is valid, the term is retained in the present statement because past usage has given it a meaning which is widely recognized and which is difficult to encompass in any other succinct term which the committee could devise. As generally understood the term carries the following implications:

1. Extensive tuberculosis of many years standing is present. This is usually bilaterally distributed.

2. Disease is reasonably well stabilized. While recurrent evidences of instability are common, these are usually rather minor and transient.

3. Chronic cavitation is commonly present and is often bilateral. Pathologically, the remaining tissue changes are deemed to be predominantly fibrotic with necrotic foci limited in size, generally well circumscribed, and not in the stage of rapid liquefaction. Emphysema is a common but not necessary component.

4. Cough and expectoration are variable but common symptoms. The general condition of the patient may be substandard, but is usually reasonably good and is often very good. Systemic symptoms are absent or, at most, are occasional and transient. Limited respiratory reserve is the rule, but marked dyspnea is not present.

5. Tubercle bacilli are present in the sputum or gastric specimens either constantly or intermittently.

6. Patients who may be included in the "Good Chronic" category vary considerably in their amenability to treatment, in the amount and kind of therapy already received, and in prognosis. Some physicians use the term without connotations on these points. In many hospitals and clinics, however, the term carries the added implication that the patient is not readily amenable to cure, at least bacteriologically, by therapeutic procedures in current usage, and that prolonged bed rest and other appropriate therapy have already been tried and have failed to achieve the goal of "inactive" tuberculosis.

II. THE PROBLEM

The problem is world-wide and well known. Some of the salient aspects are:

1. In some instances the patient is reluctant to accept hospitalization and treatment. Such a patient may feel well and be quite unconcerned by the "cigarette cough" or "chronic bronchitis" which he has had for years.

2. For most patients and for their families, a prolonged incapacitating illness becomes increasingly burdensome, especially as hope of recovery dwindles. The burden has many facets, among which the economic and the psychologic are prominent. As a result, discouragement, boredom, and resentment are common patient reactions. Such patients frequently leave sanatoriums and hospitals against medical advice; others remain, often unwillingly, and exert a disturbing and discouraging influence upon other patients.

3. A hospital bed may be occupied for years by one "Good Chronic" patient. Hospital facilities are absorbed which, if not so encumbered, could serve for the successful treatment and cure of several patients with less refractory types of tuberculosis. In this way patients with extensive chronic tuberculosis constitute a major obstacle to efficient utilization of tuberculosis hospital facilities.

4. It follows that patients in this category entail a considerable financial burden upon the community.

5. On the other hand, failure to hospitalize these patients, or discharging them back home and into the community creates other problems, for example:

(a) The patients are generally infectious and may transmit tuberculosis to others.
(b) Many remain well clinically only under very sheltered conditions.
(c) As a result, working capacity and ability to obtain satisfactory employment are extremely limited.

III. THE SOLUTION

No really satisfactory solution to the over-all problem has been devised. The best attack lies in prophylaxis, in the form of continued efforts to find tuberculosis early, before it has become extensive and chronic, and to hospitalize promptly and to treat adequately all patients with active disease. Present experience clearly demonstrates, however, that such prophylaxis is still far from a level of efficiency which is likely to eliminate the problem of the "Good Chronic" case in the immedaitely foreseeable future.

As to the existing problem, no single plan of attack is equally applicable to all patients or to all situations. Individualization is as necessary in the management of "Good Chronics" as in the management of any other tuberculous patients. This belief in individualization is implicit throughout the following generalizations:

A. Home care: The question of whether patients with "Good Chronic" tuberculosis should be kept in the tuberculosis hospital or handled at home must certainly be decided individually for each case. Prominent among the elements which deserve to influence this decision are:

1. Medical and surgical considerations affecting the particular patient at hand
2. The economic circumstances, the intelligence, and the attitudes of the patient and his family
3. Circumstances in the community.

Even in a community with plenty of tuberculosis beds it can hardly be expected that *every* patient with chronic tuberculosis and positive sputum be hospitalized indefinitely. For example, the intelligent and relatively asymptomatic elderly patient, unsuitable for additional therapy other than rest, retired on a pension and living in a home without children, can remain at home without excessive danger to himself or to others. The irresponsible bar fly, who finally persuades the tavern owner to let him wait on table or work in the kitchen to help pay for his drinks, clearly needs continued institutionalization of some type. Even where tuberculosis beds are limited, and when there is little to offer therapeutically, it is justifiable to use every reasonable means to confine such a patient to a sanatorium or hospital indefinitely.

For the usual "Good Chronic" who falls between these two extremes, the adequacy of beds in the community is bound to be one factor with an important influence upon the decision made. For most such cases, treatment at home is not the best way to attack the problem for the reasons listed in item 5 of Section II.

B. Special institutions: Special institutions and special sheltered communities have been proposed and actually tried for patients in this category. The advantages, as compared with the regular tuberculosis hospital, are:

1. Less medical and nursing care need be provided.
2. It is feasible to allow the patients more privileges and freedom of action, thus making a prolonged stay more acceptable.
3. Graded amounts of productive work can be assigned, further contributing to patient acceptance and also making such a place, in part, self supporting.
4. Beds in regular tuberculosis hospitals are freed for other patients.

There can be little doubt that special institutions and communities have their place and value. Modern progress in the field of geriatrics and in the field of chronic diseases in general is well known and impressive. The Commission on Chronic Illness has recognized chronic tuberculosis as an important member of this group of illnesses in which expert rehabilitation has been shown to play such an important role. Hospitals for chronic diseases are growing in number, and great advantages might accrue if these hospitals were provided with sections for "Good Chronic" tuberculosis wherever this is feasible.

As presently constituted, special institutions and communities have had only a minor impact upon the problem. Moreover, there are several deficiencies and disadvantages to this approach:

1. Few such places exist at present. Major expenditures for their development would inevitably reduce the tuberculosis control funds available for other purposes. In most communities the facilities for the active treatment of treatable cases are still inadequate and funds are needed for their expansion. Furthermore, the present steady increase in good case-finding programs and in therapeutic resources is steadily (albeit slowly) reducing the problem of chronic tuberculosis.

2. Few patients are willing to accept transfer to a special institution when this implies that reasonable hope for definitive cure has been abandoned.

3. Few competent tuberculosis physicians are willing to confine their work, or even to devote a major portion of it, solely to patients with extensive chronic disease. The experience of the physician in training should include tuberculosis of all types.

4. Removal of "Good Chronics" from competent medical supervision removes the most important factor which may lead to eventual cure and rehabilitation of a significant number of such patients.

C. Care in a regular tuberculosis hospital: In spite of the recognized disadvantages, continued hospitalization in a regular tuberculosis hospital or sanatorium appears still to be the procedure of choice in the management of the great majority of "Good Chronics." This does not preclude the assignment of these patients to certain wards or sections of the hospital where medical and nursing care is less intensive than on other wards, where complete self-care is the rule, where special privileges can be granted, and where special activities in the field of occupational and diversional therapy and rehabilitation can be conducted. These wards should, however, be an integral part of the regular hospital, with systematic supervision by the regular medical and nursing staffs. The reasons are:

1. The prompt recognition and treatment of exacerbations of tuberculosis are important if further deterioration in the patient's status is to be avoided. These patients also need competent medical care of collateral diseases and of intercurrent illnesses.

2. The patient who is not amenable to successful treatment today may become amenable tomorrow. This may result from gradual spontaneous improvement of the patient, which eventually makes the application of some existing therapeutic procedure feasible.

It may result from new therapeutic developments. The recent advances which have contributed most to potential salvage are:

(a) Chemotherapy, especially prolonged combined chemotherapy with streptomycin and PAS
(b) Continued improvement of surgical techniques adapted to the needs of individual patients
(c) The increased use of physiologic studies of respiratory function which have permitted more accurate judgment of the ability of a patient to tolerate a contemplated collapse or resection procedure.

This is not to suggest that recent advances in therapy have by any means eliminated the problem. Large numbers of patients with extensive chronic tuberculosis, whether clinical status is good or bad, are still beyond the reach of all therapy now available. But potentialities for salvage are already considerable, and it is impossible to predict to what extent these potentialities may be increased by future developments. Certainly efficient utilization of present and future resources requires constant medical supervision of these patients by men familiar with new developments in the field. Furthermore, modern treatment of any type of tuberculosis requires team work, to which the physician, the surgeon, various laboratory workers, and the rehabilitation team all make an important contribution. Such team work is seen at its best in the well-integrated tuberculosis hospital.

IV. CONCLUSION

The principal objective in the management of patients with "Good Chronic" tuberculosis is the actual cure and rehabilitation of as large a proportion as possible. Collateral problems should, in the majority of instances, be subordinated to this objective. The proportion amenable to cure is growing steadily as new therapeutic attacks are developed, and there is good reason to hope that this proportion will grow still larger as progress in therapy continues. The full advantages of this trend are most likely to be realized in the regular tuberculosis sanatorium or hospital. While other alternatives frequently deserve consideration in individual instances, the best general policy is continued hospitalization of patients with "Good Chronic" tuberculosis in regular tuberculosis units, hospitals, or sanatoriums.

R. O. Canada
David T. Carr
Robert H. Ebert
Kirby S. Howlett, Jr.
William M. M. Kirby
Roger S. Mitchell
William M. Peck
Norman J. Wilson
William B. Tucker, *Chairman*

Production and Distribution of BCG Vaccine in the U.S.A.

At the request of the Council of the American Trudeau Society, the Director of Medical Research asked the director of each of the three principal laboratories concerned with the production of BCG vaccine in this country for a statement on the conditions under which BCG vaccine is furnished to others by his organization. The following statements have been approved by the three directors concerned, and are printed for the interest of the members of the American Trudeau Society as an indication of the availability of BCG vaccine in the United States.

Department of Health, State of New York, Albany 1, New York

BCG vaccine prepared by the Department of Health of the State of New York is distributed within the state to health departments, medical colleges and teaching hospitals, to the superintendents of tuberculosis hospitals and clinics, and to additional hospitals and institutions in which the personnel is known to the Department to be well prepared to use it properly. Its use is recommended in groups occupationally exposed, such as nurses, medical students, and hospital personnel. It is used almost universally among these groups at present. It is also recommended for population groups with high tuberculosis morbidity and mortality rates, and where an exposure to tuberculosis has been known to have occurred or is likely to occur. In addition, large amounts of the vaccine have been supplied for study programs in the United States and in foreign countries.

Henry Phipps Institute, University of Pennsylvania, Philadelphia 47, Pennsylvania

The production of BCG vaccine at the Henry Phipps Institute is sponsored by the Bureau of Tuberculosis Control of the Department of Health of the Commonwealth of Pennsylvania. The vaccine is available without charge to any licensed physician in Pennsylvania for use on selected cases and immunization of medical personnel. Except in unusual circumstances, it is not furnished to physicians outside of the state of Pennsylvania. In view of previous relationships a certain amount has been furnished in recent years to a number of hospitals of the U. S. Indian Service. It has been provided without charge also for experimental studies in a few cities where personal arrangements were made some years ago, including the health departments of a small number of large cities where carefully conducted experimental studies are under way.

The primary purpose in preparation of BCG vaccine at the Henry Phipps Institute is research on preparation and standardization of the vaccine. It is not planned to request licensure for the manufacture of BCG vaccine or its distribution outside of the state.

Research Foundation, 70 West Hubbard Street, Chicago 10, Illinois

Any medical group or lay medical organization which has a tuberculosis program or will inaugurate one is eligible to receive BCG vaccine from the Research Foundation under the policy outlined in the pamphlet *BCG Vaccination*. This pamphlet is distributed by the Research Foundation and is available on request. It recommends vaccination of the groups listed by the American Trudeau Society as particularly appropriate for BCG vaccination (Am. Rev. Tuberc., 1949, *60*, 681). The pamphlet also lists contraindications for vaccination, techniques for administration, special considerations in the vaccination of certain groups, and the procedure for ordering the vaccine.

Research Foundation releases BCG vaccine to a limited number of private physicians

for individual vaccinations upon application and agreement (a) to pursue BCG vaccination among their patients in harmony with the spirit of the stated policy of the American Trudeau Society, and (b) to follow vaccination cases closely and report experiences and findings to Research Foundation. For purposes of animal experimentation, BCG vaccine is supplied free of charge to any reputable university or hospital.

Research Foundation is licensed for interstate distribution of BCG vaccine. Both fresh liquid and freeze-dried forms of the vaccine are supplied.

Current Status of Isonicotinic Acid Hydrazide in the Treatment of Tuberculosis

The Executive Committee of the American Trudeau Society has reviewed the available evidence on the antituberculous activity of isonicotinic acid hydrazide as presented by Hoffmann-La Roche, Inc., E. R. Squibb & Sons, and investigators cooperating with them. On the basis of this evidence, the Committee makes the following statement for the guidance of the medical profession:

1. *Chemical Structure:* Isonicotinic acid hydrazide[1,2] is a chemically pure, synthetically produced substance of the general formula $C_6H_7N_3O$.[1] It is obtained in almost colorless crystals which are highly soluble in water. A closely related derivative, which is also being studied for its antituberculous properties, is the isopropyl derivative.[1,3] Isonicotinic acid hydrazide is also related to pyrazinamide[1,4] and to amithiozone.[1,5]

2. *Activity in Vitro:* Isonicotinic acid hydrazide is bacteriostatic *in vitro* against *M. tuberculosis* H37Rv in a concentration as low as 0.02 to 0.06 γ per ml. It apparently has a very narrow antibacterial spectrum, being ineffective *in vitro* against the common gram-negative and gram-positive pathogenic bacteria, against certain protozoa, and against the influenza virus in mice. It may possess some slight antifungal properties.

3. *Activity in Vivo:* In several species of experimental animals (mice, guinea pigs, rabbits, and monkeys), experiments on the effectiveness of isonicotinic acid hydrazide against tuberculous infection with virulent human strains of *M. tuberculosis* have given promising results in arresting the course of the experimentally produced disease. On the basis of these observations, isonicotinic acid hydrazide appears to be approximately the therapeutic equivalent of streptomycin; at least in the first few months of treatment. Observations on the emergence of strains of tubercle bacilli which may be resistant to isonicotinic acid hydrazide, either *in vitro* or *in vivo*, are meager, and it is not known if such strains will emerge during treatment or if such emergence will have therapeutic significance. A definite increase in resistance has been obtained *in vitro* with one strain (BCG).

4. *Toxicity and Pharmacology:* Although the toxicity of isonicotinic acid hydrazide has been determined fairly accurately in several species of animals, some aspects of the pharmacology and toxicology of the drug have not been completely elucidated. On the basis of available studies it appears that both isonicotinic acid hydrazide and its isopropyl derivative are of relatively low toxicity in dosage ranges which appear to be effective. The drugs are apparently largely excreted in the urine. Within an hour or so after administration they appear to be well distributed throughout the body (blood serum, cerebrospinal fluid, pleural fluid).

5. *Dosage:* On the basis of preliminary studies, the indicated daily dosage is in the range of 3 to 5 mg. per kg. of body weight (150 to 300 mg. per day for the average adult). This dosage is given by mouth in two or three divided doses. The drug may also be given parenterally.

6. *Toxicity in Man:* In the dosage range indicated, preliminary observations in man indicate that there is little significant or serious toxicity. The following have been ob-

[1] See structural formula.

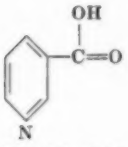
[2] "Nydrazid," proprietary name of E. R. Squibb & Sons; "Rimifon," proprietary name of Hoffmann-La Roche, Inc.

[3] "Marsilid," proprietary name of Hoffmann-La Roche, Inc.

[4] "Aldinamide," proprietary name of American Cyanamid Company.

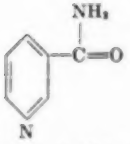
[5] "Tibione," proprietary name of Schenley Laboratories.

STRUCTURAL FORMULAS OF NICOTINIC ACID DERIVATIVES



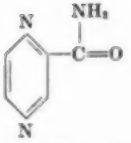
Nicotinic Acid

Isonicotinic Acid

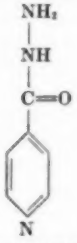


Nicotinic Acid Amide

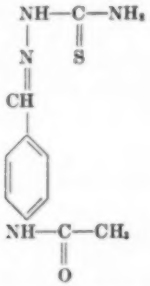
Isonicotinic Acid Amide



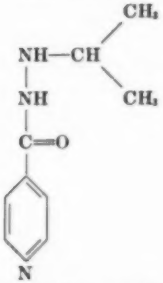
Pyrazinamide, proprietary name of Lederle Laboratories, "Aldinamide"



Isonicotinic Acid Hydrazide, proprietary name of E. R. Squibb & Sons, "Nydrazid"; Hoffman-La Roche, Inc., "Rimifon"



Amithiozone, proprietary name of Schenley Laboratories, "Tibione"



1-Isonicotinyl-2-Isopropyl hydrazine, proprietary name of Hoffmann-La Roche, Inc., "Marsilid"

served but on a more or less transitory basis even though drug administration is continued:

a. Constipation
b. Difficulty in starting micturition (in males especially)
c. Increased reflexes
d. Positional hypotension and dizziness
e. Eosinophilia (in about 10 per cent of cases)
f. Slight drop (0.5–1.0 gm.) in hemoglobin concentration
g. Occasional casts and traces of albumin and reducing substances in the urine.

Toxic effects on the eighth cranial nerve, impairment of renal or hepatic functions, or dermatologic manifestations associated with the drug have not been observed so far.

7. *Activity in Man:* Preliminary observations on the effect of isonicotinic acid hydrazide on the course of tuberculosis in man have been limited largely to patients with far-advanced pulmonary disease, extensive tissue destruction, positive sputum and, as a rule, considerable symptomatology, many of whom have failed to respond or would not be expected to respond to other available therapy. In such patients, treated with 3 to 5 mg. per kg. per day for up to five months of therapy (the majority treated for two to three months), the following changes in clinical course have been observed:

a. Reduction in fever, if present, in two to three weeks, in the majority
b. Reduction in cough, in the volume of sputum, and in the number of tubercle bacilli raised (as determined by smear). No information is available on conversion of the sputum as determined by culture.
c. Gains in appetite, weight, and strength
d. Some clearing of the reversible component of the pulmonary tuberculous disease by roentgenographic observation
e. Initial favorable response has been observed in such non-pulmonary lesions as draining sinuses and fistulas, mucous membrane tuberculosis, and in a very few cases of miliary and meningeal tuberculosis.

8. *Problems:* At the present time complete information is lacking on many aspects of the therapy of tuberculosis with isonicotinic acid hydrazide. Among the unknowns are the following:

a. The mechanism of action of the drug on the tubercle bacillus—whether it is tuberculocidal or tuberculostatic; the effect upon the enzyme chemistry of the tubercle bacillus, et cetera
b. The mechanism of action upon the host—basically, the precise toxicity in man
c. The optimal dosage—the number of milligrams per day; whether it needs to be given every day; the optimal mode of administration
d. The duration of therapy—whether its effect is comparable to that of streptomycin and para-aminosalicylic acid (PAS), indicating relatively long courses of treatment, or whether shorter courses may be as effective
e. The rate of emergence of drug-resistant strains of tubercle bacilli
f. The effect of the drug upon the bacteriology of the patient—data are lacking on conversion of sputum by culture; the tissue bacteriology after varying amounts of treatment will need to be studied.
g. The question of potential relapse after initial improvement
h. The question of whether basic systemic therapy of tuberculosis (especially bed rest) can be modified as a result of treatment with isonicotinic acid hydrazide.

9. *Precautions:* At present there is no reason to believe that the fundamentals of therapy of tuberculosis should be altered in any way when isonicotinic acid hydrazide is employed. Patients receiving the drug should be hospitalized for careful observation. They should be studied in institutions where potential toxic manifestations may be watched for most carefully and where effects upon the course of the underlying tuberculosis may be carefully observed so that suitable alterations of therapy may be initiated when indicated. Routine laboratory precautions should include frequent blood counts and urinalyses, neurologic examinations, and tests for renal and hepatic insufficiency.

10. *In General:* The introduction of a new drug in the therapy of tuberculosis is likely to raise more questions for a few years than it will answer. There is no knowledge at the present time that isonicotinic acid hydrazide or its isopropyl derivative will accomplish more than has been accomplished with streptomycin and PAS. It may prove to be an additional drug of great value. It may be years before its exact contribution to the therapy of tuberculosis can be assessed accurately. A large reservoir of undetected and untreated cases of active tuberculosis exists throughout the United States, and there is every expectation that, in spite of the more effective chemotherapy of tuberculosis currently available, the need for hospitalization in institutions with qualified personnel and adequate laboratory facilities will increase rather than decrease. There is at present no basis for expecting that isonicotinic acid hydrazide, or any other drug available, can safely be counted upon to reduce the duration of hospitalization. Rather, in most instances, at least, it may lead to prolongation of hospital treatment since effective chemotherapy may facilitate desirable forms of therapy not otherwise possible.

It should be emphasized strongly that, with more numerous effective antituberculous compounds available in the treatment of tuberculosis, more intensive case finding than ever will be indicated. Only through this means can maximum advantage be taken of improvements in therapy.

11. *Summary:* After a review of available data on the action of isonicotinic acid hydrazide and its isopropyl derivative upon the tubercle bacillus *in vitro*, and upon the course of experimental tuberculosis in animals and clinical tuberculosis in man, it may be stated that their demonstrated action, although highly encouraging, appears in no way to alter the basic principles of the treatment of tuberculosis as presently understood. Much more work will need to be done to ascertain the exact place of these drugs in the treatment of the disease. With several carefully coordinated studies in prospect, it is anticipated that further information will accumulate rapidly.

March 5, 1952

NOTES

THIRD COMMONWEALTH AND EMPIRE HEALTH AND TUBERCULOSIS CONFERENCE

The Third Commonwealth and Empire Health and Tuberculosis Conference arranged by the National Association for the Prevention of Tuberculosis will be held in London from July 8 to July 13, 1952. The conference is open to all who are interested in preventive medicine, industrial executives, nurses, social workers, health administrators, members of public authorities, regional hospital boards, et cetera. At the last conference in 1949, more than a thousand delegates from fifty countries were present, and representatives

from Australia, Belgium, Canada, Fiji, France, Gold Coast, India, Jugoslavia, New Zealand, South Africa, Trinidad, and the U. S. A. have already arranged to attend this year.

Interesting subjects for discussion include "Protective Vaccination," "Contemporary Ideas in the Management of the Tuberculous Patient," "The Social Worker and the Tuberculous Family," "The Patient in Industry," and "Tuberculosis in British Colonial Territories." Among those who have already promised to speak are Dr. G. C. Brink, Department of Health, Ontario, Canada; Dr. B. A. Dormer, Chief Tuberculosis Officer, Union of South Africa; Dr. J. B. McDougall, World Health Organization; and Dr. T. Holmes Sellors, Thoracic Surgeon, London.

A new feature of the Conference will be special smaller gatherings for those with particular interests, whether doctors, nurses, public health officials, or social workers, at which appropriate subjects will be discussed. Receptions for delegates have been arranged at many well-known institutions, and a large number of visits to hospitals and sanatoriums have been organized in addition to tours of general interest.

All information can be obtained from the National Association for the Prevention of Tuberculosis, Tavistock House North, Tavistock Square, London, W. C. 1.

NEW APPOINTMENT

Dr. James E. Perkins, Managing Director of the National Tuberculosis Association, has announced the appointment of Mr. Frank W. Webster to the position of Field Director on the staff of the American Trudeau Society. With headquarters in the office at 1790 Broadway, New York, N. Y., Mr. Webster will assist with all activities of the American Trudeau Society and will maintain close coordination with its state and regional sections.

Mr. Webster, who is at present the Executive Secretary of the North Carolina Tuberculosis Association, Inc., will begin his new duties July 1.

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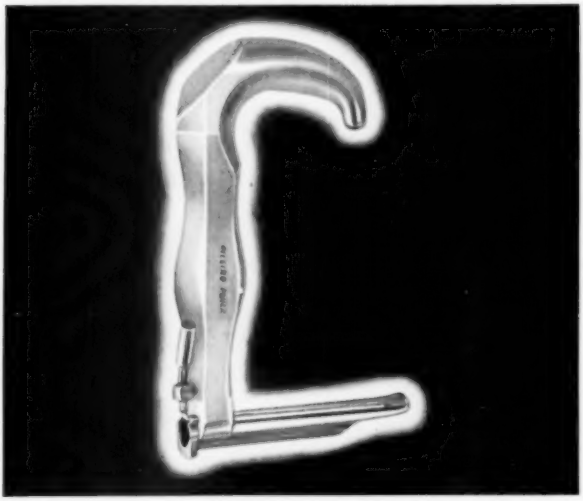

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(2 days after discontinuance of Dihydrostreptomycin) Considerable clearing of acute exudative process in the diseased lung; cavity smaller and wall thinner.

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NATIONAL TUBERCULOSIS ASSOCIATION

ANNUAL MEETING ***MAY 26–29, 1952***

BOSTON, MASSACHUSETTS

The annual meeting of the National Tuberculosis Association will be held in Boston, Massachusetts, May 26–29, 1952. The Statler Hotel will be headquarters.

Some 1500 rooms have been promised by the hotels listed below for the accommodation of those attending the meeting. Arrange to share a twin bed room if possible.

Application for room reservations should be made directly with the hotel of your choice.

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Bradford	275 Tremont Street	$5.25- $7.50	$8.80- $11.00
Copley Plaza	Copley Square	$5.84- $7.85	$9.90- $14.00
Copley Square	Copley Square	$4.00	$7.00- $8.80
Kenmore	Kenmore Square	$5.50- $8.50	$9.50- $12.50
Lenox	Exeter Street	$4.50- $5.50	$9.00
Manger	North Station	$4.75- $7.75	$8.00- $10.50
Parker House	School Street	$6.00- $8.00	$10.00-$14.00
Pioneer	410 Stuart Street	$3.50- $3.75	$5.50- $7.00
Shelton	91 Bay State Road	$5.00- $7.00	$8.00- $10.00
Somerset	400 Commonwealth Ave.	$7.00- $8.00	$12.00
Statler	Park Square	$6.00-$10.00	$12.00-$16.00
Touraine	62 Boylston Street	$5.00- $7.00	$9.00- $12.00
Vendome	160 Commonwealth Ave.	$4.00- $8.00	$7.00- $10.00
University Club	40 Trinity Place	$6.00	$8.00

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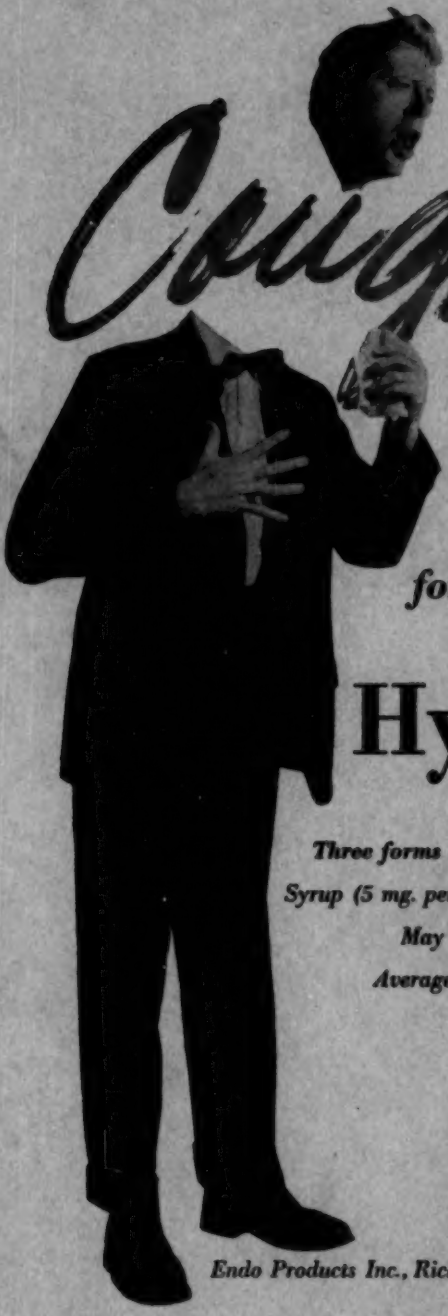

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